A biochemical and modelling study of the life history of Antarctic krill, Euphausia superba.

Thesis

A biochemical and modelling study of the life history of Antarctic krill, *Euphausia superba*

A thesis submitted in accordance with the requirements of the Open University for the degree of

Doctor of Philosophy

by

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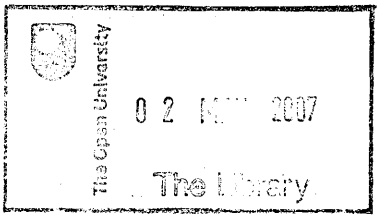

Abstract

This thesis investigates various aspects of the energetics of Antarctic krill, *Euphausia superba.* In the first section, proximate chemical and elemental composition is examined in relation to life history, environmental variability and physiology. It is shown that life-history stage has a significant effect on both proximate and elemental composition. Krill growth in the field was found to differ markedly between individuals, so the observed variability in chemical composition is investigated with respect to differing growth rates in an attempt to derive a predictive relationship between elemental composition and growth in krill. It is shown that elemental composition cannot provide a proxy for short term variations in growth rate. A method is then developed which applied modified stoichiometric theory to krill to determine proximate composition from elemental composition data. It is possible to predict realistic chemical composition for all life history stages from elemental composition data which reflects that directly measured.

In the second section, an energy budget model for Antarctic krill is developed, incorporating a novel representation of the energetic costs of growth. This model is then used to explore key aspects of the life history of krill (growth, maturity and reproductive success) under a range of environmental conditions. The model produced an ecologically realistic simulation of growth and reproduction in female krill. These simulations indicated that food availability and temperature have a highly significant role in determining the life history of krill, with a potentially significant knock-on effect to the Southern Ocean ecosystem. Results of the model predict an average growth rate of 0.05 mm d^{-1}, but that in times of scarce food temperature has a significant effect on growth. A significant benefit to the animal occurs from being in cold waters during periods of limited food.

Acknowledgements

This PhD has been a great (and mostly enjoyable) experience and for that I have to thank my supervisors for coming up with this project and giving me this opportunity. Firstly, Andy Clarke deserves many thanks, for his initial encouragement on the road to this PhD, and subsequently for his time, patience, valuable advice and encouragement throughout its duration. Eugene Murphy has been invaluable in teaching me about modelling from scratch (in a way my non-mathematical brain could cope with) and providing much appreciated advice and discussion on all aspects of this PhD. The late Bob Boutilier provided initial discussions and encouragement, with John Spicer very generously stepping in with advice, support and constructive comments part way through the project.

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Chapter 1 Introduction

THE SOUTHERN OCEAN

Antarctica is a unique continent, covering an area of 13.8 million km^2 (including nearly one million km^2 of permanent ice sheet), covered by 25.4 million km^3 of ice. The coastline of Antarctic is 45,000 km long, of which only 5,500 km is rock; the rest being ice (18,800 km of ice shelf and 21,000 km ice covered coast) (Antarctic Digital Database, British Antarctic Survey).

The Southern Ocean which surrounds the Antarctic continent is a unique, fascinating and challenging environment, characterised by low temperatures, high latitudes, pronounced seasonality and unique oceanography. The waters of the Southern Ocean account for nearly 10% of the world's oceans (Constable et al., 2003), some 35 million km^2 (Mackintosh, 1972a), but are effectively separated from adjacent water masses by the Polar Front at its northern, circumpolar boundary, which leads to very distinct physical and biological characteristics. Since the first heroic age expeditions to the Antarctic, there has been great interest in the Southern Ocean, stimulated originally by a fascination with the abundant and charismatic wildlife observed in its waters. This early interest quickly expanded to represent a huge range of multidisciplinary interests, starting with the groundbreaking early scientific expeditions of *HMS. Challenger* and *RRS. Discovery* and continuing into recent times with modern scientific expeditions such as BIOMASS (Biological Investigations of Marine Antarctic Systems and Stocks) and JGOFS (Joint Global Ocean Flux Study).

Antarctic waters display considerable spatial and temporal variability in their physical and chemical parameters (Stein & Heywood, 1994). The general circulation of the Southern Ocean has been well described (Siedler et al., 2001) and can be broadly summarised: prevailing strong westerly winds in the lower latitudes of the southern

hemisphere act on the water surrounding the almost circular continent of Antarctica, forming an easterly flowing current, the Antarctic Circumpolar Current (ACC); the strongest surface current in the world, which flows unbroken around the globe (Orsi et al., 1995) (Figure 1.1). It is this current which forms the effective barriers with adjacent oceans. Two major fronts are found within the ACC; the Sub Antarctic and Polar Fronts (Peterson & Whitworth, 1989), with the southerly Polar Front often taken to be the nominal boundary of Antarctic polar waters. A second major current exists, flowing westwards adjacent to the continental margin, driven by coastal easterly winds, the Antarctic Coastal Current, also known as the East Wind Drift Current (Tchernia & Jeannin, 1980; Fahrbach et al., 1992). This westerly current is interrupted by several clockwise gyres which allows some interchange between the two major bodies of water (Miller & Hampton, 1989).

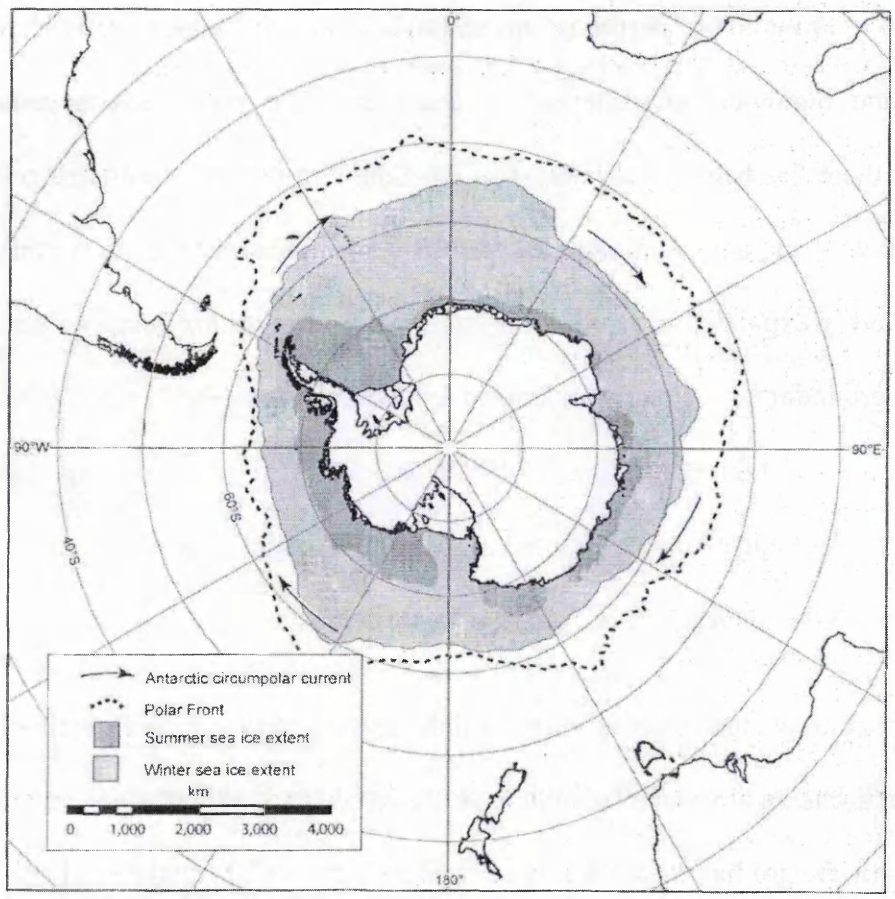

Figure 1.1. The Antarctic continent, showing the extent of summer and winter sea ice and the approximate positions of the Polar Front and the Antarctic Circumpolar Current.

In contrast to the northern polar regions, the Southern Ocean is subject to seasonal freezing at much lower latitudes. A large area of the Antarctic surface waters is covered by annually forming sea ice, in summer about 4 million km^2 of ocean are frozen, rising dramatically to 20 million km^2 in the winter (Zwally et al., 1983) (Figure 1.1). The sea ice cover acts as an insulator, reducing the transfer of heat between the ocean and the atmosphere, also reducing the amount of sunlight penetrating the water. As the ice begins to melt, it mixes with the previously insulated waters, lowering the water temperature and stratifying the water column. This annual advance and retreat of sea ice is one of the defining physical features of the Southern Ocean.

As well as the shading effect of sea ice, the amount of available light is dictated by the seasonal changes in day length; at the higher latitudes there can be almost total darkness in winter and near constant daylight during summer; a major feature in the pronounced seasonality of the Antarctic. Southern Ocean waters are cold, with a small temperature range of between -2 and 5° C at the extremes; waters close to the continent can be close to -1.9° C year round (Clarke, 1987a) (although recent studies have shown that even these waters show small summer increases in temperature (Barnes et al., in press)), whilst temperatures at South Georgia can range from -1 in the winter to 5° C in the summer (Whitehouse et al., 1996).

Until recently it was believed that the Southern Ocean was a region with universally high primary production, to be able to support such biological abundance observed in coastal regions. However, it has been subsequently shown to be one of the world's major oceanic High Nutrient – Low Chlorophyll (HNLC) regions. This means that the Antarctic waters are an area of low overall productivity; off shelf waters are HNLC and may be the least productive ice free waters anywhere, with primary production at 16 g C m^{-2} yr^{-1} (Priddle et al., 1994). High productivity is patchy and confined to marginal sea ice

zones, frontal systems and shelf waters; with these regions being responsible for 40% of total primary production in the Southern Ocean.

Productivity in these regions is highly variable, for example in the Georgia Basin to the north west of South Georgia blooms of up to 15–20 mg chlorophyll *a* m^{-3} have been observed on a number of occasions, with mean values of ≥3 mg m^{-3}; yet waters to the east of the island are more typical of HNLC waters with mean chlorophyll *a* concentrations an order of magnitude smaller (< 0.3 mg m^{-3}) (Korb & Whitehouse, 2004). Annual carbon production figures for this region have been given as 30–40 g C m^{-2} yr^{-1}. Recent estimates by Arrigo et al. (1998a) using monthly climatological phytoplankton pigment concentrations from the coastal zone colour scanner (CZCS) calculate an annual primary production in the Southern Ocean of 4414 Tg C m^{-2} yr^{-1}; between four and five times greater than previous estimates made using in situ data. Their daily carbon primary production estimates range from 0.075 g C m^{-2} d^{-1} in the pelagic province of the Weddell Sea in June to 3.94 g C m^{-2} d^{-1} on the shelf of the Ross Sea in December. Peak production rates were observed during December or January in all areas, with the highest daily production rates associated with the continental shelf; the maximum monthly average rate of production on-shelf was over four times the pelagic maximum.

It is clear that the Southern Ocean is heterogeneous in terms of production (and presumably also zooplankton). Various schemes have been devised to divide up the Southern Ocean: Gloersen et al. (1992) define simple geographic sectors by longitude, Longhurst et al. (1995) base their zones on ocean colour, fronts and regional oceanography, Tréguer and Jacques (1992) divide the Southern Ocean into zones based on sea ice dynamics and Arrigo et al. (1998a) suggest division into three ecological zones defined by sea ice coverage and bathymetry (the pelagic province, marginal ice zone and the shelf).

Because of these unique and variable characteristics, the Southern Ocean ecosystem is one of the best areas for studying large-scale oceanographic processes. Several major large-scale studies have employed both multinational and multidisciplinary efforts to examine the pronounced interannual variability in physical and environmental factors and help elucidate the links between this variability and that observed in the biology. The BIOMASS programme of SCAR (the Scientific Committee for Antarctic Research) first recognised the need to concurrently study the physical, environmental and biological marine environment if the temporal variations in distribution, abundance, behaviour and productivity were to be understood (Stein & Heywood, 1994). The BIOMASS programme was followed by JGOFS and the Southern Ocean Global Ocean Ecosystem Dynamics (SO-GLOBEC) study. Other smaller scale year-round monitoring programmes also exist, such as the RACER (Research on Antarctic Coastal Ecosystem Rates) and ROAVVERS (Research on Ocean-Atmosphere Variability and Ecosystem Response in the Ross Sea) programmes of the Palmer Long Term Ecological Research (Palmer LTER) project study and the Rothera oceanographic Time Series (RaTS) carried out by the British Antarctic Survey (BAS). All of these studies have predominantly concentrated on the Atlantic sector and summaries of their findings can be found in El-Sayed (1994), Fasham (2003) and Smith & Anderson (2003).

So, it is the unique character and focus for concentrated research that makes the Southern Ocean such an excellent model for investigating the links between regional marine ecosystems and large-scale biological and physical processes (Quetin et al., 1994). Discussions in this thesis are limited to the Atlantic sector of the Southern Ocean, with a particular focus on South Georgia and the Scotia Sea.

IMPORTANCE OF KRILL IN THE SOUTHERN OCEAN FOOD WEB

The Southern Ocean ecosystem is relatively simple in terms of food web interactions and can arguably be considered to be self contained. It is characterised by the abundance and diversity of top predators it supports, including whales, seals, penguins, fish and albatross. The previously held paradigm was that the food chain could be simply represented by three trophic levels: diatoms, krill and consumers, however it is now widely accepted that the Southern Ocean food web is more complex (Clarke, 1985). The importance of bacterial and microbial components in the food web is now recognised (Karl et al., 1991; Delille, 2004), krill have been shown to be omnivorous (Price et al., 1988) and other primary consumers; copepods, salps and the amphipod *Themisto gaudichaudii* can, in some areas, occupy the same fundamental trophic role as krill (Bocher et al., 2001; Pakhomov et al., 2002).

However, Antarctic krill, *Euphausia superba,* remain central to the functioning of the Southern Ocean ecosystem. They are among the world's most abundant zooplankton species and can form the largest observed aggregations of marine life on the planet (Mangel & Nicol, 2000). In the waters around South Georgia, krill contribute 50% of the total zooplankton biomass (Atkinson et al., 2001). This dominance of biomass is an important factor in their position as a key species in the food web. Krill form a direct link between the primary production of the phytoplankton and the large top predators of the Antarctic seas; comprising the main component of the diet of marine mammals and seabirds in the area, which form some of the largest breeding aggregations in the world (Croxall et al., 1988).

Although the Southern Ocean food web is similar in fundamental structure to those elsewhere, in some places at some times, the system is dominated by the energy flux through krill. This includes the Scotia Sea, the area of study for this thesis. The huge biomass of krill in the Southern Ocean has attracted interest as a resource for potential

human exploitation. The fishery for krill is relatively recent; exploratory fishing was in progress from the late 1960s (Everson & Miller, 1994), but it was not until the early 1970s that a commercial fishery became active (Ichii, 2000). From there the fishery expanded, reaching a peak in the early 1980s, with nine countries operating krill fisheries (Suzuki & Shibata, 1990) with a catch of over half a million tonnes reported for the 1981-2 season. The fishery has been regulated by CCAMLR (Commission for the Conservation of Antarctic Marine Living Resources) since 1982, with current catches of 80,000 tonnes (Ichii, 2000) (2% of the 4 million tonnes total allowable catch for CCAMLR Area 48 in 2000, in which all krill fishing occurred in that year). The Atlantic sector is subject to the greatest fishing effort, with the largest catches predominantly around South Georgia and the South Orkneys (Everson & Miller, 1994). Commercial uses of krill drive the demand for the fishery and they include human consumption, aquaculture and domestic animal feed and pharmaceuticals (see reviews in Nicol & Endo, 1999; and Everson, 2000a).

Because of their critical role in the Southern Ocean food web and the importance of krill as a commercially exploited resource, knowledge of their ecology is central to understanding the dynamic functioning of the ecosystem and in developing advice for the management of the fishery.

KRILL ECOLOGY

Euphausia superba (Dana), are one of 85 species of euphausiid; a group of crustaceans which are an important part of the pelagic zooplankton community worldwide. Antarctic krill have a circumpolar distribution, south of the Polar Front and are typically found close to the continental shelf (Mackintosh, 1972b) in the top 200m of the water column. It is frequently found aggregated in swarms, which can range in size (from 9 to 1800m wide) and shape (small round or oval aggregations to large, irregular, elongated swarms) (Everson & Miller, 1994). However, it is the small, compound swarms that are more commonly observed (Watkins et al., 1986), although larger swarms are seen to form

in regions with high current shear. Superswarms are rare, but can be up to 150km diameter and 100–250m in depth (Everson & Miller, 1994). Although disputed, it is generally thought that Antarctic krill display diurnal migration behaviour, typically spending the night at ~20m, days at an average of 136m (Siegel & Kalinowski, 1994) with a lower density and greater swarm length at night.

Krill have a comparatively long lifecycle of 5–7 years (Ikeda et al., 1983; Nicol et al., 1991), reaching maturity within 2–3 years. Larval development takes approximately 8 to 10 months after the eggs hatch (Ross et al., 1996). Larvae undergo a developmental ascent after hatching at depth, reaching the surface as stage calyptosis 1 of approximately 2 mm in length, and ready to start feeding. Larvae continue to grow throughout the summer, moulting into early furcilia by the end of the summer season. Growth and maturation continue during early winter, with late furcilia stages predominating by August, when growth is suspended until spring. In October growth begins again and the larvae moult into the juvenile stage at around 20 mm in length. Growth continues to adulthood through a range of maturity stages, with the rate of this development dependant on environmental factors during that particular season (e.g. food availability, temperature). The maximum length obtained by fully mature adults is around 60 mm, although it can take several years to reach this size, during which time the krill may have undergone annual periods of seasonal shrinkage during winter months (Ikeda & Dixon, 1982; McGaffin et al., 2002).

There has been much research undertaken on krill over many years, partly as a result of the increasing interest in the potential exploitation of Antarctic marine living resources. This large volume of work covers all aspects of krill behaviour, distribution, physiology and chemical composition. Figure 1.2 shows that research interest in krill over the years has not become focused on any particular field of research, rather interest continues in krill as a subject for a wide range of disciplines.

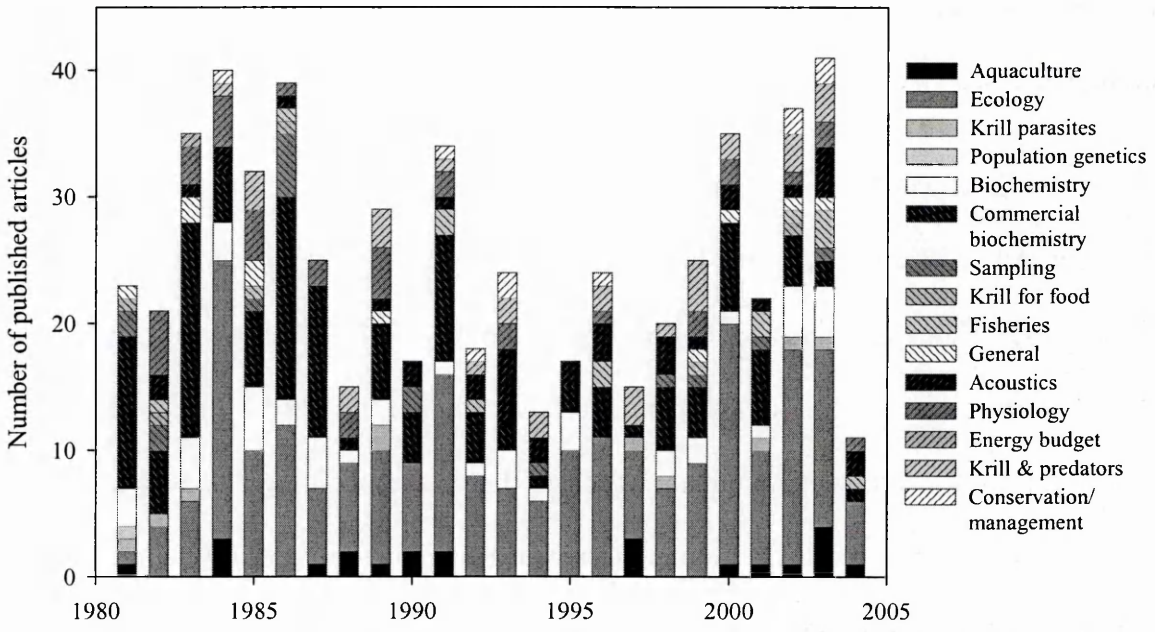

Figure 1.2. The number and subject of primary research and reviews published on *Euphausia superba* as listed on Web of Science from 1981-2004.

There have been two major peaks in krill research: the *Discovery* investigations of the 1930s and the BIOMASS investigations begun in the 1970s. The *Discovery* investigations between 1926 and 1940 intensively studied the Southern Ocean, providing much of our current knowledge including the most comprehensive study on the ecology of krill (Marr, 1962). The principle objective of the BIOMASS programme was *'to gather a deeper understanding of the structure and dynamic functioning of the marine Antarctic ecosystem as a basis for the future management of potential living resources'* (BIOMASS 1977) (Siegel & Kalinowski, 1994). Emphasis was on elements of the Southern Ocean ecosystem that were believed to contribute most to the general energy flow or were a priority for exploitation and as such krill was the subject of much targeted research (El-Sayed, 1994). BIOMASS added fundamental studies on the biochemistry and physiology of krill as well as furthering the ecological studies of Marr (1962), improving knowledge of distribution and abundance. As a result of the BIOMASS programme critical aspects of krill ecology of which our knowledge and understanding remained poor were highlighted, which has since resulted in much targeted work utilising the great expertise and techniques

developed during the BIOMASS research. The increase in interest in krill as a source of protein in aquaculture, human food source and resource for biochemical applications has driven the research on further.

Whilst it would be impossible to summarise the huge body of work in the literature here, several major reviews of the biology of krill exist. The original and classic work by Marr (1962) is an excellent source of basic krill biology, from which all subsequent work has developed. Mauchline (1980a) produced a complete review of the biology of all species of euphausiid, which contains much of interest concerning *Euphausia superba.* The volume 'Euphausiacea Bibliography: a world literature survey' (McWhinnie et al., 1981) comprehensively documented the current literature at that time. Miller and Hampton (1989) produced a major review of recent developments in krill biology, which included work from the BIOMASS programme and focused on topics pertinent to the effective management of krill exploitation. The BIOMASS summary volume (El-Sayed, 1994) further documented the advancements made by this programme and the most recent review by Everson (2000a) concentrated mainly on aspects of the biology pertinent to krill fisheries and stock management. There also exist many special volumes of journals dedicated to the study of krill biology, often summarising conferences or workshops (e.g. Journal of Crustacean Biology 4, special issue (1984), Canadian Journal of Fisheries and Aquatic Sciences 57 (2000) and Marine and Freshwater Behaviour and Physiology. 36 (2003)).

Despite this large volume of focused work on the species over many years, there is much of the biology of Antarctic krill which remains unknown. Arguably, the most important aspect for understanding the responses of krill to its environment, namely a detailed understanding of its energetics, is the area that still has the most questions to be answered. Knowledge of an animal's energy budget is essential for understanding its role and function in the ecosystem, and without adequately functioning energy budgets, we are

hampered in our efforts to construct effective ecosystem models, an essential tool in modern ecological studies.

Several studies have made significant progress towards the goal of a predictive, functioning energy budget for krill (Clarke & Morris, 1983; Miller & Hampton, 1989; Quetin et al., 1994; Atkinson et al., 2002; Meyer et al., 2003). However, these studies have all been based around the traditional formulation of the energy budget:

$$C = P + R + U + F$$

where: C = total food or energy intake; P = production (somatic and reproductive); R = respiration; U = excretion; and F = faeces (Clarke, 1987b).

This has its origins in work done for the International Biological Programme (IBP) (Blaxter, 1967) predominantly on domesticated animals and was based on experimentally observed biological responses and not on the underlying physiology driving these responses. There are several limitations to this form of the energy budget: in the current model key physiological process which cost the animal significant amounts of energy are absent and it is difficult to account for locomotor costs, e.g. swimming in zooplankton. It is arguably more meaningful to consider the production of new tissue separately for reproductive and somatic tissue as rates of synthesis are different for each type of tissue and are limited by different constraints. The costs to the animal of synthesising each type of tissue will be different as they have a differing biochemical composition. Pelagic invertebrates like krill, have to swim continuously (and hence expend energy) to maintain their position in the water column, a cost over and above the strict definition of basal metabolism. Feeding also has a metabolic cost to the animal – specific dynamic action – which must also be accounted for. It is therefore more ecologically meaningful to consider metabolic rate (O_2 consumption) as divided into several independent, but interlinked components: respiratory cost of feeding, swimming, production of somatic and production

of reproductive tissue on top of the traditional idea of basal metabolism. This approach, proposed by Clarke, has been discussed in detail (Clarke, 1987b; Quetin et al., 1994; Clarke, in prep.) but has not yet been applied to a functioning energy budget for any organism.

The development of such novel approach to dynamic energy budget modelling would allow us to develop detailed predictive models of krill energetics which can be used to examine an organism's response to environmental variability at a detailed scale. Previous energetic models for krill have been constructed (Hofmann & Lascara, 2000; Alonzo & Mangel, 2001; Fach et al., 2002) and used to address pertinent ecological questions, however until we can be confident that we are adequately predicting energetic demands, we must exercise caution in applying these models to large scale ecosystem models – the ultimate goal of such research.

It is important, when considering energetics of krill, to consider how the organisms partition incoming energy. The key to understand this is in examining the underlying chemical composition of krill, both proximate and elemental, and how this varies with life-history. By shedding light on the relationship between chemical and elemental composition and the changes with life-history we will understand the factors that determine the chemical composition of krill and gain a better understanding of how metabolic demands are affected by the underlying biochemical make up of the animal.

Therefore, the overall aims of this study are to:

- determine the detailed chemical and elemental composition of a range of life-history stages of Antarctic krill, *Euphausia superba*;
- investigate whether this chemical composition can be related to observed growth rates;

- derive a stoichiometrically consistent composition for krill;
- develop and test an energetic model for krill growth.

Chapter 2 Sampling methods

The sampling for this project was conducted over two field seasons, during oceanographic research cruises which formed part of a British Antarctic Survey (BAS) core science programme. The DYNAMOE (Dynamics and Management of Ocean Ecosystems) programme uses the Southern Ocean ecosystem as a model system with which to examine the globally important question of how regional marine ecosystems are connected to large-scale biological and physical processes. Ultimately, an understanding of these complex interactions should allow us to predict the implications of human impacts, both direct (e.g. fisheries) and indirect (such as climate change), on the ecosystem and attempt to influence management where possible. The DYNAMOE programme focuses on the South Georgia ecosystem, its links in the wider Scotia Sea ecosystem, and considers the implications for the whole of the Southern Ocean. It is undertaking fundamental research to determine the key interactions and the dominant biological - physical links in the ecosystem and elucidate which aspects of ecosystem function are crucial to successful prediction and management.

A key component of the DYNAMOE programme is the fieldwork, which takes the form of a series of annual multidisciplinary research cruises, each addressing a specific question in addition to gathering long-term environmental and biological monitoring data. The field work component of this doctoral study used two of these research cruises as a sampling platform. Both cruises were undertaken on the British Antarctic Survey's *RRS James Clark Ross*; a multi-disciplinary oceanographic research vessel specially designed for scientific operation in polar waters. In this chapter, I will first outline the overall aims and context of the two cruises, before describing my own work on board.

CRUISE JR70: FLUX AND MARINE PRODUCTION EXPERIMENT

Cruise JR70 was conducted between 1st January and 6th February 2002, with the aim of determining the relative importance of flux and local production in maintaining krill and zooplankton populations at South Georgia. The main study area comprised the Western Core Box (WCB) located to the north of Bird Island at the western end of South Georgia (Figure 2.1); an area sampled biannually to investigate interannual variability in krill biomass and environmental conditions.

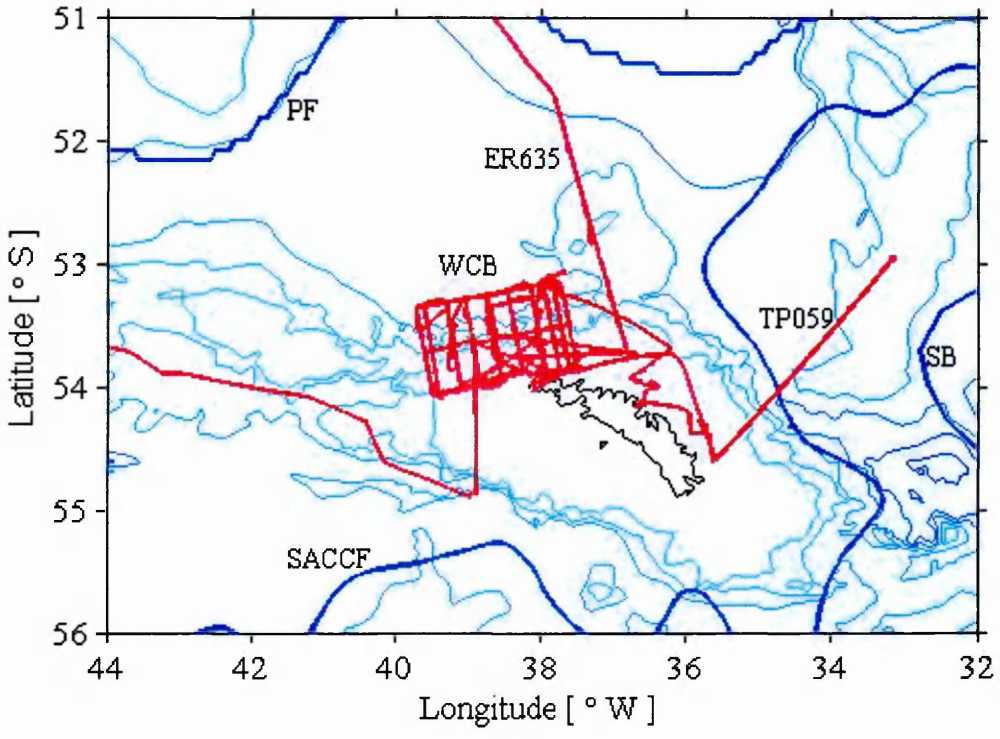

Figure 2.1. Cruise track for JR70 around South Georgia, showing the Western Core Box (WCB), the long transect surveys (ER635 and TP059) and the approximate position of the Polar Front (PF), Southern Boundary (SB) and Southern Antarctic Circumpolar Current Front (SACCF).

The focus of investigation for this cruise was a conceptual model describing inputs into and outputs from the WCB (Figure 2.2). The model assumed that processes occurring within the core box would affect krill biomass such that observed changes in biomass between the beginning and end of the study would be the product of what is transported

into the box, what comes out of the box, growth (production) within the box and the predator demand.

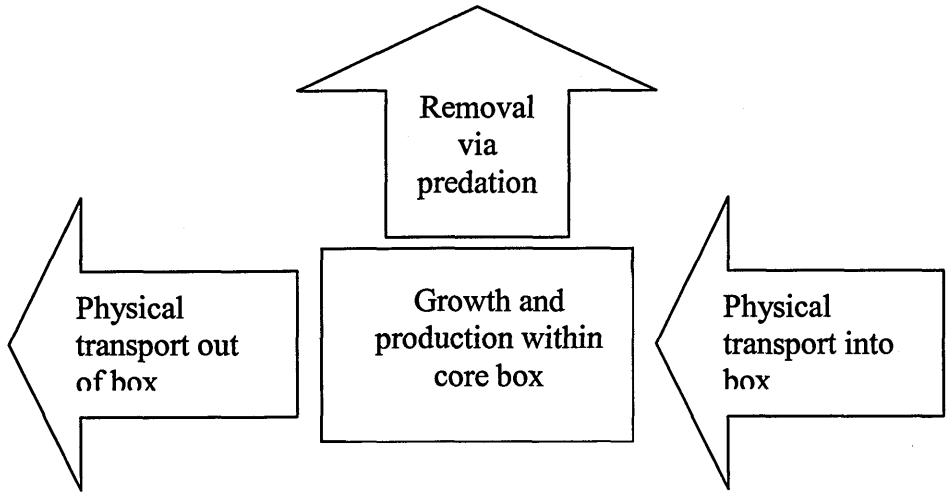

Figure 2.2. Conceptual model of the processes affecting krill biomass, the focus for the work carried out during the JR70 cruise (Ward et al., 2002).

A range of sampling techniques were employed to gather the data necessary to develop this model; CTD deployments, ADCP, satellite tracked drogued drifters, phytoplankton and nutrient analysis, zooplankton biomass estimates (both acoustically and by quantitative net hauls), zooplankton community characterisation, copepod and krill production and growth experiments and predator observations.

The cruise sampling area concentrated on the WCB, with the addition of two long transect surveys (ER635 and TP059, Figure 2.1) following the track of a satellite altimeter over the continental shelf and continuing off shore for 240 km. Zooplankton sampling was conducted throughout the cruise duration by a variety of methods, enabling krill collection from throughout the sampling area (Ward et al., 2002).

CRUISE JR82: LARGE SCALE DISTRIBUTION IN THE SCOTIA SEA

Cruise JR82 was undertaken in the Scotia Sea between 7th January and 20th February 2003, contributing to the UK GLOBEC programme as a component to the international Southern Ocean GLOBEC in addition to the DYNAMOE programme core research. The principle aims of JR82 were to investigate large-scale transport, growth and survival of krill across the Scotia Sea. A key gap in our understanding of the South Georgia ecosystem is the nature of the supply of krill to the region: South Georgia is far to the north of the typical habitat of krill in the seasonal ice zone and does not experience the regular sea ice which is often cited as being essential to the survival and over wintering of Antarctic krill (Mackintosh, 1972a; Quetin & Ross, 2001; Brierley et al., 2002), yet krill are abundant. However, despite this abundance, small krill (body length c 15–25 mm) are typically absent from the South Georgia population. It is hypothesised that krill do not reproduce successfully here and are instead transported into the region from the ice zone in the Scotia Sea towards South Georgia. To fully understand the mechanisms that supply the krill population at South Georgia, aspects of the physical transport of water and growth and survival of krill across the Scotia Sea must be investigated. JR82 followed 8 long transects across the Scotia Sea, from north of the Southern Antarctic Circumpolar Current Front (SACCF) to about 63° S, or the sea ice edge, whichever was reached first (Figure 2.3). The standard survey area of the WCB was also sampled, allowing for a comparison between the two years of sampling.

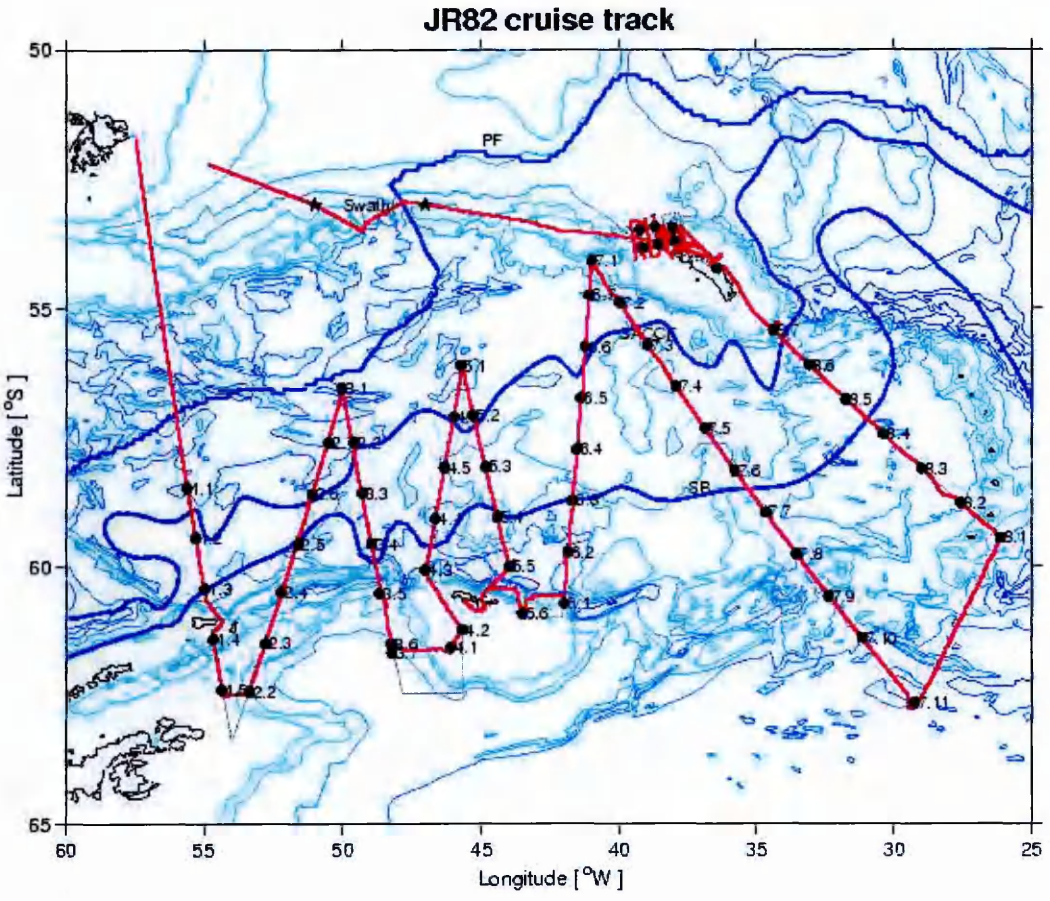

Figure 2.3. Cruise track for JR82, showing the approximate position of the Polar Front (PF), Southern Boundary (SB) and Southern Antarctic Circumpolar Current Front (SACCF). Sampling stations shown as bold circles.

MY WORK ON THE CRUISES

The majority of krill preserved for this study were collected during targeted krill fishing events using a Rectangular Midwater Trawl (RMT 8). The RMT 8 is a quantitative net with a mouth area of eight square meters. Although not originally designed for krill fishing (Clarke, 1969), it has been routinely used by BAS in its krill sampling programmes since the early 1970's. The RMT used on these cruises has two discrete nets, which can be opened and closed by an electronic release gear mounted on the net bridle. This enables the selective fishing of two discrete swarms in one haul, subsamples of the same swarm, or samples at different depths within a krill layer to be taken.

Targeted krill fishing was carried out mainly at night as the krill were typically nearer the surface than in the day and the effects of net avoidance were minimized

(Everson & Bone, 1986). A krill swarm was located in an approximate predetermined sampling position using a hull mounted echosounder (Simrad EK 500 during JR70, Simrad EK 60 on JR82) operating at 38, 120 and 200 kHz (Figure 2.4). The bridge was contacted and the position of the swarm marked on the ship's navigation system. The ship would then turn around and position itself for a second pass through the mark indicating the suspected position of the swarm. During the repositioning of the ship, the net was readied on deck for deployment and once the ship was on course the net was deployed over the aft gantry and winch wire paid out to position the net at the approximate position of the target swarm (allowances are made to compensate for the speed of the ship, typically 2.5 to 3 knots when fishing, and the upwards movement of the net in the water when opened). The RMT was triggered to open one of its nets once the target swarm was sighted on the echosounder to be passing under the ship. After a period of time the net was closed and the second net triggered to open at a time and position dependant on the method of fishing required at that particular station.

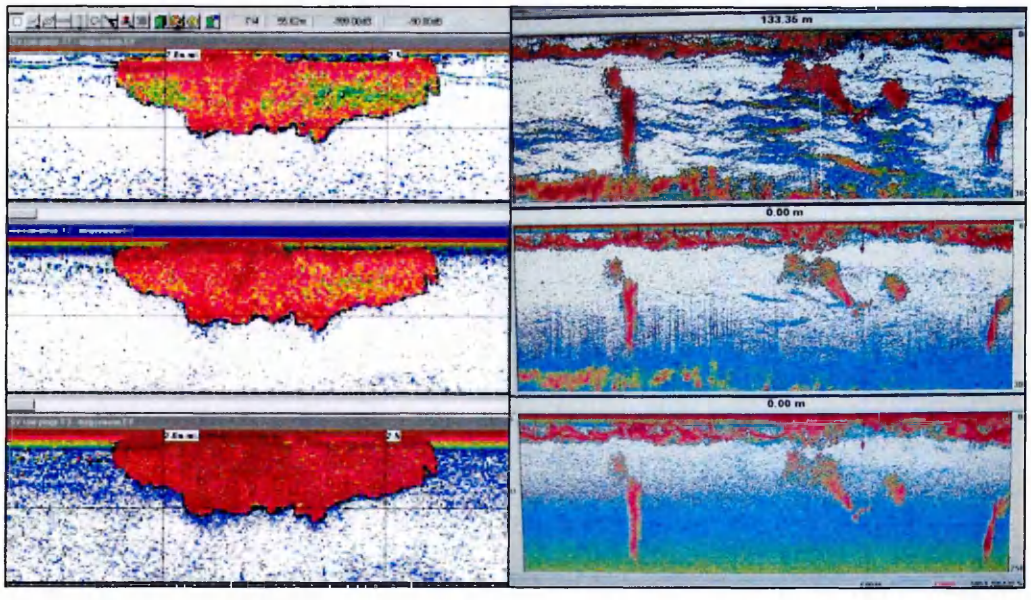

Figure 2.4. Examples of echograms showing krill swarms. Left, extensive krill swarm seen in the company of whales during JR82 (Atkinson et al., 2003), vertical scale 100m. Right, example of typical krill swarms encountered during JR70 showing the surface layer and distinct krill swarms below, vertical scale 300. The three screens show the three different frequencies used by the echosounder; 38, 120 and 200 kHz from top to bottom. Red shows the strongest backscatter.

In order to retrieve the krill in the best possible physiological condition for on-board experimentation, non-filtering cod-end buckets were used and haul duration was kept below 20 minutes. Nets were recovered on board and freshly caught krill were immediately transferred to 150 litre circular bins containing freshly collected sea-water and held in a cold room maintained at the local ambient temperature. From these bins, healthy krill were selected by the various workers requiring krill for on-board experimentation. Any remaining krill were available for the work reported in this thesis.

Small numbers of healthy, freely swimming krill were selected one at a time from the 150 litre bin and transferred to the laboratory in freshly collected, ambient temperature seawater. Individual krill were measured (see page 23) and sex and maturity stage was determined (see page 25). Each krill was then blotted dry on tissue paper to remove any excess seawater, placed individually in a scintillation vial and immediately frozen at -80° C. The freezing of krill separately in scintillation vials allowed the samples to be easily tracked and identified on return to the UK and prevented damage and loss of delicate appendages that can occur if samples are preserved together.

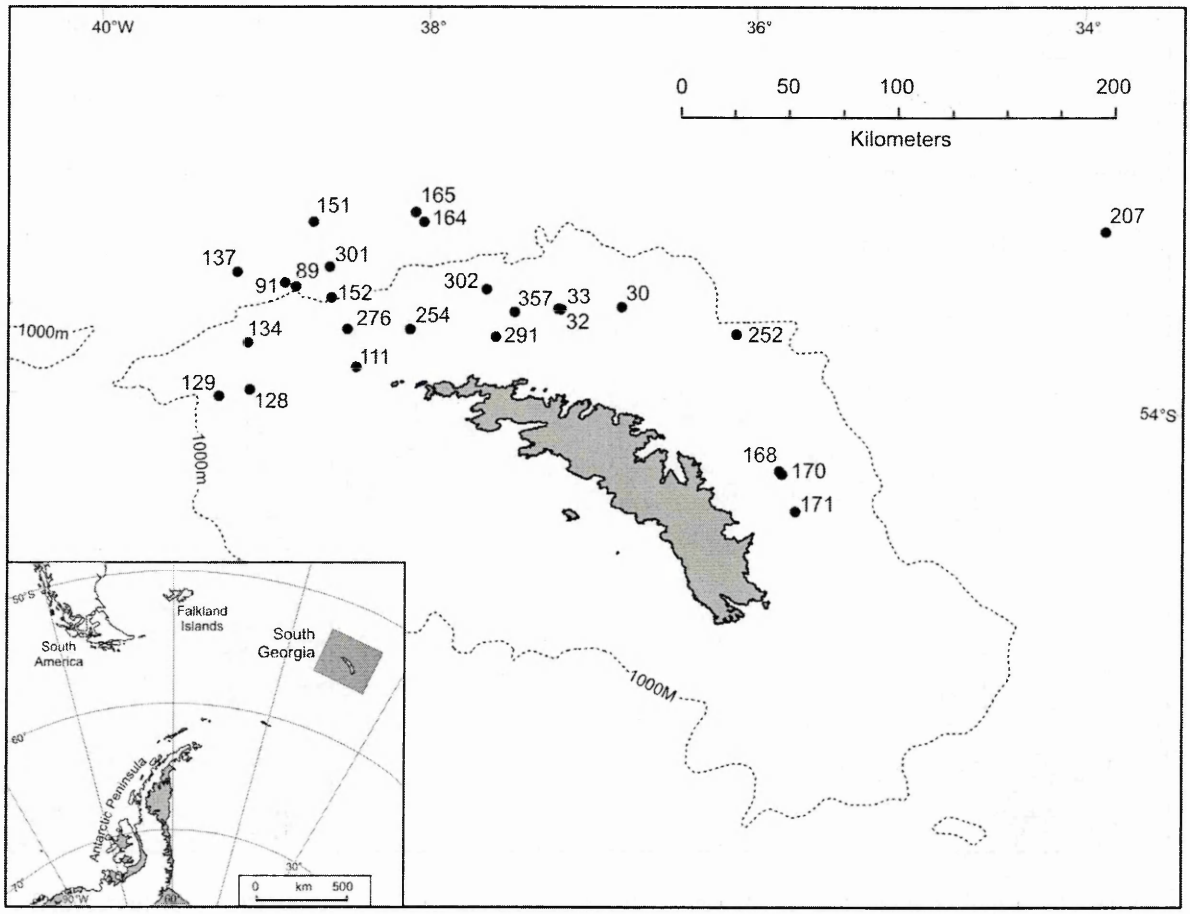

Figure 2.5. Sampling stations on JR70 where krill were preserved for this study. Dotted lines represent the continental shelf (1000m depth contour).

Occasionally, krill caught in good condition by other zooplankton sampling methods such as the bongo or neuston nets were opportunistically preserved. The selection of krill for preservation was not random, instead attempts were made to collect as broad a range of sizes and sexual maturity stages from as many geographically spaced sampling events as possible from the krill available. Figure 2.5 and Figure 2.6 show the sampling stations from where krill used in this study were collected.

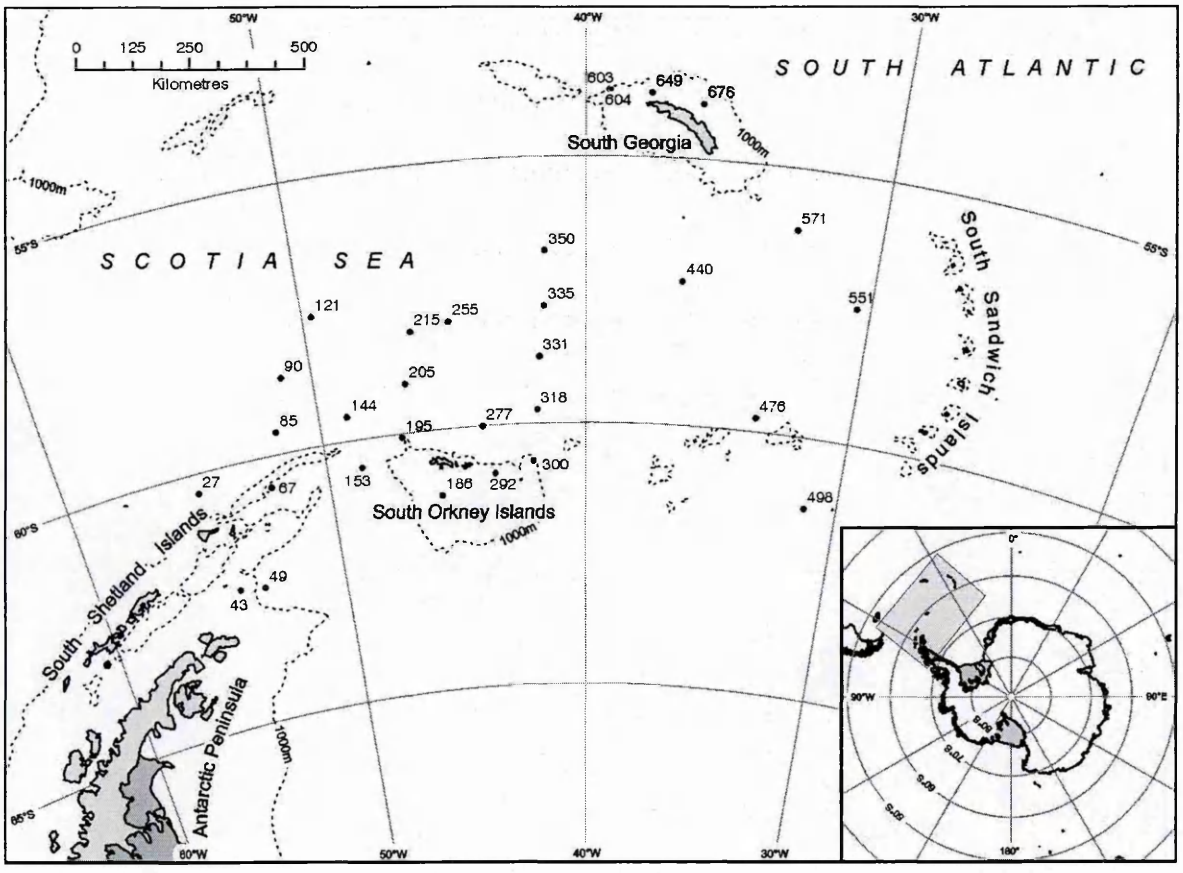

Figure 2.6. Sampling stations on JR82 where krill were preserved for this study. Dotted lines represent the continental shelf (1000m depth contour).

Samples were returned frozen to the UK on board the ship, and transferred to the -80°C storage facility at BAS Cambridge until required for analysis. Material was frozen for between 2–12 months prior to analysis.

Body length measurement

There are various standards for measuring the length of krill described in the literature, with different methods adopted by different groups of researchers. There are several practical difficulties in measuring krill precisely: they may have become damaged in the nets, loosing fragile external features; measurements are often made on board ship in moving laboratories; measurements made on live animals can be problematic as they are inclined to 'kick' and not lie straight along the ruler; and the measurement of preserved samples can be complicated by the fact that shrinkage can occur under some preservation methods (Lockyer, 1973; Miller, 1983). Some of the published methods for length measurements are as follows (see Figure 2.7):

- Reference length (RF): Mauchline in his review of length measurements in Antarctic krill (1980b) suggested a reference length of '*the lateral distance between the anterior lateral edge of the carapace and the posterior end of the uropods, excluding the terminal setae'*. However, this method, although not including any easily damaged appendages, requires the measurement to be made under a microscope which is not always practical or readily available at sea.

- Standard Measure 1 (S1): Mauchline (1980b) suggested a more practical measurement of total length '*the lateral or dorsal distance between the anterior tip of the rostrum and the posterior end of the uropods, excluding the terminal setae'* defined as Standard Measure 1. This definition of length is used by the Australians, but others workers from Japan and the USA use the tip of the telson as the posterior measurement (Everson, 2000b). The position of the end of the uropods and the tip of the telson is roughly equivalent in Antarctic krill. This method may not be suitable if the rostrum has been damaged during sampling.

- *Discovery* measurement (AT): Lockyer (1973) describes the method used to record the length of krill sampled by the *Discovery* expeditions of 1926-1936, given as '*the length from the anterior margin of the eye to the tip of the telson*'. This method has been adopted by workers from Germany, South Africa and the UK (Everson, 2000b). It has also been adopted by CCAMLR in their scientific observer programme (CCAMLR, 1999) as the most practical measurement for work at sea. However, care must be taken with this measurement that the eye does not move forward on its stalk as the animal is placed on the ruler, especially when measuring live animals.

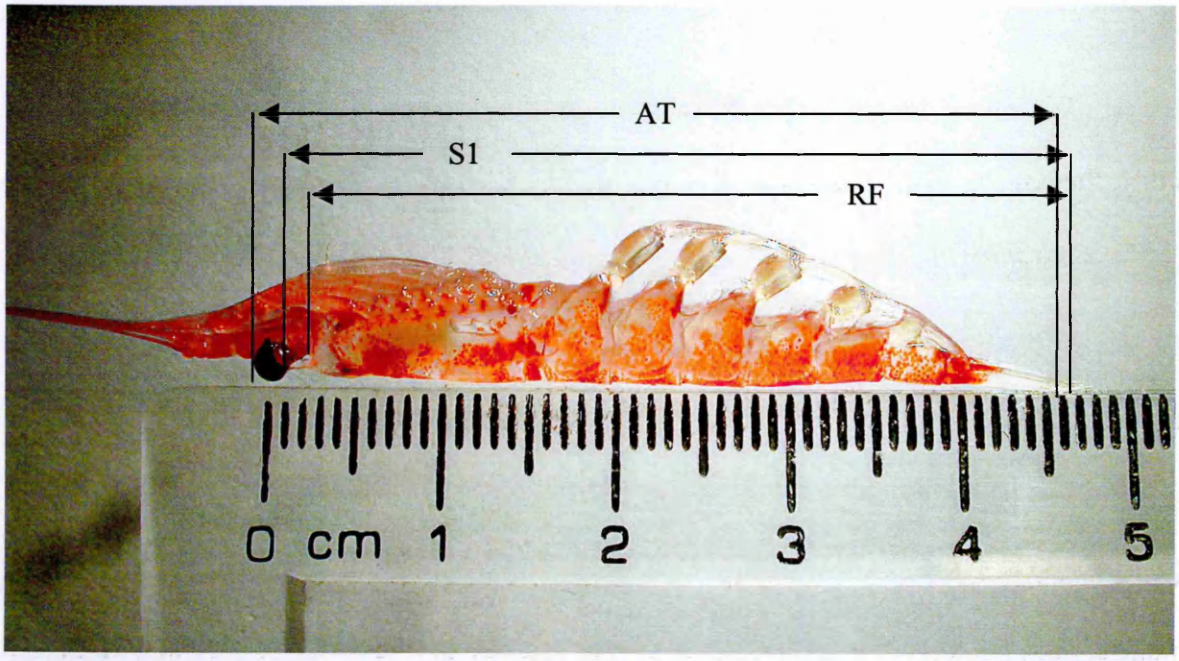

Figure 2.7. The three main methods for measuring krill length (after Everson, 2000b). Note that in this example, the eye has moved forward on its stalk during the process of photography and is no longer in the correct position for the measurement of AT length (eye to telson) as recorded (it should be level with 0 mm on the ruler shown).

Other measurements can be used if the animals are damaged, and these measure to points on the abdomen, or the carapace only. A review of a wide selection of these measurements and the relationships between them can be found in Morris et al. (1988). Carapace measurements are commonly used in studies of predator diet where krill may not

be preserved intact (Hill, 1990), although carapace length is more accurately related to maturity rather than total length (Färber-Lorda, 1994).

In this study, the *Discovery* measurement (AT) was used for all krill sampled. This was chosen as it is the method adopted by fellow workers from BAS, it is the CCAMLR standard and it is easy to determine at sea on a large number of live samples without excessive handling. Krill were measured live under a magnifying lamp on a clear 15cm ruler, marked with 1 mm divisions (Figure 2.7). Care was taken to ensure the dorsal side of the krill was aligned flat along the edge of the ruler and that the eye had not moved forward on its stalk at point of measurement. The same ruler was used for both cruises to ensure consistency and lengths were rounded down to the nearest whole mm (Lockyer 1973). When comparing length measurements from the literature with data from this study, the conversion equations of Morris et al. (1988) were used standardise lengths to the AT measurement.

Sex and Maturity Stages

For this study it was important to accurately determine the sex and maturity stage of each individual krill. It is relatively easy to distinguish visually mature male and female krill: males generally have a leaner shape with wider abdominal segments and are more intensely pigmented. Males have conspicuous ejaculatory ducts on the ventral side of the thorax that can be seen as a pair of red oval shaped patches. Females, when mature, have a conspicuous structure (the thelycum) on the ventral, posterior region of the thorax which is bright red in colour. Compared to mature males, females have a proportionally larger carapace and thinner abdomen, and when gravid have a dramatically swollen thorax. It is not possible, however to distinguish more detailed classifications by eye and the identification of juveniles, immature adults and discrete adult maturity stages needs more careful examination under magnification. Under low power, it can be seen that males have developed modified endopods of the first pair of pleopods (Makarov & Denys, 1981), the

petasmae, which are used to transfer spermatophores from the male to the female. The stage of development of the petasmae in males and thelycum in females are indicators of maturity stage.

There exist several schemes to classify sexual maturity in krill, although some are much more detailed and contain finer subdivision than others. Studies such as Bargmann (1937) and Cuzin-Roudy and Amsler (1991) are very detailed and allow description of not only maturity stage, but position in the spawning cycle. However, these studies require histological investigation and are therefore unsuitable for ship-board studies, or studies such as that reported here, where the animals need to be preserved intact. Instead, a scheme based on external morphology is required that can classify individuals quickly and reliably, without damage, during shipboard studies. One such scheme is the classification of Makarov and Denys (1981) which has been widely used and is adopted by CCAMLR for their Scientific Observer Programme.

In this study, sex and maturity stage were determined according to Makarov and Denys (1981), under low power magnification (x12). For clarity and consistency with BAS standard measurements, the classification codes of Makarov and Denys were recoded with a hierarchical alphanumeric code after Morris et al. (1988), with the first letter representing sex, the second the developmental stage (sub-adult or adult) and the number the maturity stage, with increasing maturity corresponding to increasing numerical coding (the codes used are therefore as follows: J = juvenile, FS = female sub-adult, MS1–MS3 = male sub-adult, FA1–FA5 = female adult, MA1–MA2 = male adult). The detailed correspondence between the two codings and defining characteristics of each stage are shown in Table 2.1. Throughout this thesis, krill will be described according to these sex and maturity stage codes. Pooled sexual maturity stages are also referred to with MS referring to sub-adult males, MA to adult males, and FA to adult females.

Table 2.1. The correspondence between the sexual maturity stage codings of Makarov and Denys (1981) and Morris et al. (1988) and the characteristics that define these stages. Table adapted from Morris et al. (1988).

Morris et al. (1988)	Definition	Makarov and Denys (1981)	Characteristics
J	Juvenile	JI	No secondary sexual characteristics visible.
MS1	Male sub-adult stage 1	MII A (1)	Petasma single, undivided lobe.
MS2	Male sub-adult stage 2	MII A (2)	Two-lobed petasma.
MS3	Male sub-adult stage 3	MII A (3)	Petasma also has wing present.
MA1	Male adult stage 1	MIII A	Petasmae fully developed; ejaculatory ducts red.
MA2	Male adult stage 2	MIII B	Fully formed spermatophores present in ejaculatory ducts.
FS	Female sub-adult	FII B	Developing thelycum present, but colour feeble or absent.
FA1	Female adult stage 1	FIII A	Thelycum is fully developed, bright red and visible through the gills.
FA2	Female adult stage 2	FIII B	Thelycum dirty red, empty spermatophores present.
FA3	Female adult stage 3	FIII C	Developing ovary fills body cavity, but body not swollen.
FA4	Female adult stage 4	FIII D	Enlarged ovary clearly visible, thorax and first 2 abdominal segments swollen. (Gravid).
FA5	Female adult stage 5	FIII E	Thorax and first 2 abdominal segments swollen, but no enlarged ovary visible. (Post-spawn).

Chapter 3 Chemical composition of Antarctic krill

Despite a large body of literature available on the chemical composition of Antarctic krill, *Euphausia superba,* as presented in the methods section of this chapter, we still have a limited understanding of how this composition alters with growth, maturity, season and environmental conditions. In order to develop a reliable energy budget for krill, we need a better understanding of how metabolic demands are affected by the underlying biochemical make up of the animal.

By examining in detail both the proximate and elemental composition of krill across a range of sexual maturity stages and sizes, sampled from different geographical locations and subject to varying environmental conditions; we should obtain a clearer picture of the factors that determine the chemical make up of krill. By understanding how the body chemistry of krill changes as it grows, and in response to environmental variability, our understanding of the metabolic costs involved in growth, reproduction and synthesis of new body tissue will be improved, enabling better parameterisation of subsequent energy budget models.

This chapter presents the experimental results from the analytical component of this study, which will be used throughout the rest of this thesis. Proximate chemistry will be discussed in full first, followed by elemental chemistry in a second section of this chapter. These results present interesting new patterns, important to our understanding of krill energetics.

PROXIMATE CHEMISTRY

Introduction

The study of proximate chemistry is a tool used widely in studies of physiology, nutrition and ecology for a range of objectives. Because of its broad usage, there exists a wide range of methodologies for the determination of each chemical component, adapted

for specific applications. There has been much discussion of the best techniques and many authors have published reviews and comparisons (e.g. Peterson, 1983; Grémare & Vétion, 1994; Brooks et al., 1995). These methods are now well established, though in relatively few cases have the techniques been tested on, or adapted for, use with marine invertebrates.

The proximate chemical composition of krill has been investigated several times previous to this study, although no other studies include experimental results for all the components presented here, analysed in krill from one sampling season. Raymont et al. (1971) were the first to present a comprehensive proximate composition study on krill. Mauchline and Fisher (1969) and Mauchline (1980a) present reviews of the early literature, and studies by Clarke (1980) and Zhu and Wang (1989) have subsequently investigated the major chemical components. Whilst these studies agreed on the broad composition of the major components in krill, and described some differences between sexes, they did not examine the detailed composition of the range of sexual maturity stages, as presented here.

Aside from purely academic curiosity, many of the investigations in to krill chemistry have been carried out to assess its potential commercial importance; whether with an aim relating to human nutrition (Bykov, 1975) or aquaculture (Roschke, 1978; Storebakken, 1988). The increasing population of the world requires more proteins for human nutrition which lead to scientists investigating new potential sources for protein, such as krill.

Here the aim is to determine a broad chemical composition for krill (specifically protein, lipid, carbohydrate, chitin, water and nucleic acids), together with knowledge of its variability, to inform subsequent development of energy budgets but which is also consistent stoichiometrically with the elemental composition (See Chapter 5).

Methods

All proximate analyses were carried out on frozen, whole krill for several reasons; firstly, to avoid the breakdown or loss of any of the major chemical components during the process of drying (Clarke, 1980). Also, it has been shown that oven drying can lead to slightly reduced dry masses (2%) and a loss of lipid, protein, chitin and ash when compared with freeze-dried samples (Postel et al., 2000). It has often been postulated that the comparison of the amounts of each chemical component expressed as a function of wet mass is less misleading than the consideration of dry mass fractions, where the increase in one leads to an apparent decrease in another. It is worth noting however, that percentage wet mass, dry mass and g/100g are all essentially the same thing, and suffer similarly from the same problem; as one proximate component increases, others apparently decrease in response. But this is in fact just a mathematical artefact, as the mass of the remaining components may have remained constant. To counter this, many studies use some form of standard animal, which works well in cases where there is a suitable standard to use (such as an echinoid test, a molluscan shell, or length in any vertebrate whose skeleton does not shrink). Unfortunately krill have no permanent skeletal structures and the dynamic body mass changing only at moult adds confusion. As krill are ~ 80% water, any changes in chemical composition expressed as wet mass are less obvious, but it does allow for the individual changes in water content with sex, maturity stage or position in the moult cycle to be accounted for. Given these difficulties, data are presented in the thesis as percentage wet mass as the primary method, as this was what was actually measured. Percentage dry mass is also given, using an average value for water content calculated for each maturity stage grouping, and a table showing the data presented for a standard animal is given in Appendix A for completeness.

Colorimetric methods for determining concentrations of given chemicals involve the specific reaction of chemical reagents with the assay substrate in a concentration-

dependant manner, producing coloured products which can be measured spectrophotometrically. Colour development in the samples is then compared with a standard curve prepared with known standard compounds and water blanks to control for background absorbance. In this study, calibration curves included at least seven concentrations analysed in duplicate and were fitted with a least squares regression using MINITAB (Version 13, Pennsylvania State University) in all cases. All colorimetric assays involved the analysis of triplicate subsamples for each individual krill, with each subsample assayed in duplicate or triplicate depending on the assay. The mean of these subsamples was taken and is presented as the result for each individual (although some outliers were removed at this stage if they were considered to be erroneous, lying more than one standard deviation outside the range of the other replicates). All reagents were made up with MilliQ ultra pure water. All statistical analyses were performed using Students *t*-tests, one-way ANOVA and least squares regressions in MINITAB as appropriate. All data were checking for normality using the Anderson-Darling normality test and found not to deviate significantly from normal. Where Students *t*-tests were performed on data expressed as a percentage, the data were arcsine transformed prior to analysis.

Water content

Typically water content has been quantified by either drying to a constant mass in an oven, or by the process of freeze drying. Although many studies publish wet to dry mass relationships for krill, there are fewer published data giving absolute water content. Water content can be extrapolated from published allometric relationships, but these estimations are not included in the summary of water content data available for krill presented here (Table 3.1), as a summary of allometric equations is presented in Appendix B.

In the study reported here, water content was determined as the difference between frozen and dry mass. It was not possible to weigh fresh krill at sea, so frozen mass was taken to be a good approximation to fresh mass, as excess water was removed before freezing (see Chapter 2). It has been noted that some species lose significant amounts of water on freezing, although this was not thought to occur in krill preserved at -80 °C in small volume closed containers (which helps to reduce sublimation loses), and assayed within a short period. Frozen mass was determined in all JR70 samples by weighing on a balance (Mettler AT250) to ± 0.01 mg in a cool room at a temperature of 4 °C to reduce the possibility of the samples thawing significantly. Samples were weighed in small batches that were transferred into the cool room and kept in a pre-frozen cool box. Mass was determined after 30 seconds, allowing the reading to stabilise. This time period was established by weighing a solid piece of metal, a marble and an individual frozen krill and recording their mass every 10 seconds over a period of 15 minutes. In all cases there was a sharp rise over the first 20 seconds as the balance stabilised, followed by a slower decrease to a period of constant mass at 30 seconds in the metal and the marble. Although the krill continued to increase in temperature throughout the experiment, it was thought that 30 seconds was sufficient time to allow the balance reading to stabilise without the krill thawing out significantly.

In this study, dry mass was measured after the samples were dried to a constant temperature at 60 °C in a drying oven, consistent with previous studies (Clarke, 1980; Torres et al., 1994; Kirkwood, 2001). Time taken to achieve constant mass was investigated, with 20 krill dried for five days, and mass measurements taken at periodic time intervals (Figure 3.1). Krill were cooled in a desiccator before weighing on a balance to ±0.01 mg, with desiccant in the weighing chamber and with the first stable mass reading recorded (Postel et al., 2000).

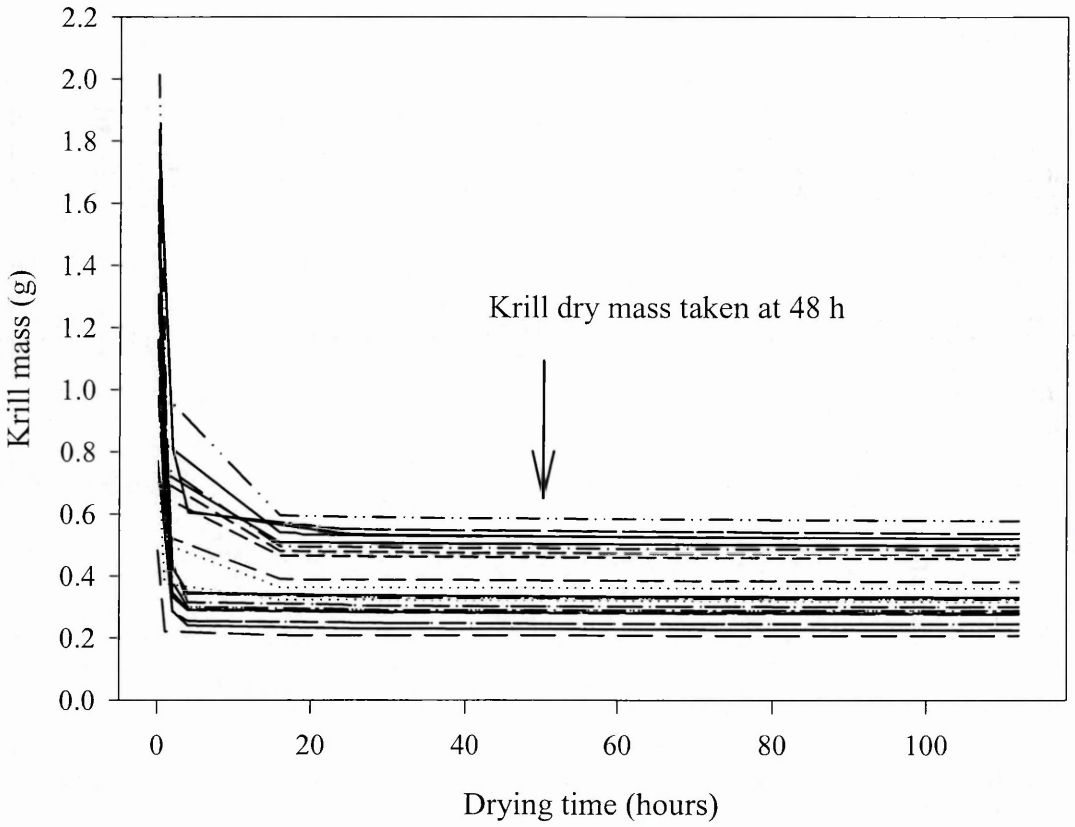

Figure 3.1. Time taken to achieve constant mass for 20 individual krill of varying length and sex.

From this experiment, it was seen that constant mass was achieved in all cases by 48 hours so this was adopted as the routine drying time throughout this study.

Table 3.1. Summary of water content data for *Euphausia superba* from the literature (% fresh mass). Measurements of whole animals unless otherwise stated. (SE, standard error; *n* = total number of krill analysed; * Juvenile, male and female, but no gravid females).

Study	Analysis method	Antarctic location	Time of yr.	Krill analysed	Mean ± SE	Range	*n*
Raymont et al. (1971)	70 °C, constant mass	Peninsula	Dec	Mostly juvenile	82.39	79.5–86.1	23
			Jan		76.76	74.8–78.0	7
Clarke (pers. comm.)	75 °C, 46 hours	Palmer Station	Dec	Male	78.29	75.71–84.25	23
			Winter	Male	73.87±0.43		14
				Male and female	76.29±0.45		15
Bykov (1975)	—	—	—	All krill	—	73.7–82.3	—
				<35 mm		80.0–82.0	—
				35–55 mm		77.6–80.5	—
Il'ichev (1965)	Chizhova instrument	—	Dec	—	83.10		—
			Jan		81.6		—
			Feb		78.8		—
			March			73.0–75.6	—
Kryuchkova and Makarov (1969b)	—	Scotia Sea	19 Feb	Female	79.1		—
				Juvenile	75.9		—
			2 March	Male	76.4		—
				Female	75.5		—
				Juvenile	75.2		—
			11 March	Male	79.3		—
				Female	75.0		—
				Juvenile	73.7		—
Clarke (1980)	60 °C, 48 hours	South Georgia	Feb	Juvenile	75.93		5–20
				Male	80.05		5–20
				Gravid females	75.95		5–20
Nicol et al. (1992a)	Freeze dried	67°46'S 75°24'E	Feb	All krill *	77.26	75.89–78.69	300
Suzuki and Shibata (1990)	—	—	Dec–Feb	—	80.8		—

Study	Analysis method	Antarctic location	Time of yr.	Krill analysed	Mean ± SE	Range	*n*
Mauchline (1980a)	Synopsis of all available data	—	—	—		73–85	—
Cullen et al. (2003)	60 °C, 24 hours. Abdominal muscle tissue	Deception Island	Nov '99	Male	83.61±0.46		7
				Female	80.37±1.68		8
			Feb '00	Male	76.43±0.44		8
				Female	76.46±0.35		10
			May '00	Male	76.51±0.33		10
				Female	77.09±0.22		9
			Nov '00	Male	81.08±1.00		7
				Female	78.95±0.60		9
Sidhu et al. (1970)	Vacuum oven at 80 °C	South of Tasman Sea	31 May	Juvenile	74.3		—
		North of the Ross Sea	6 April	Juvenile	73.2		—
Ikeda and Kirkwood (1989)	Freeze dried	Off Enderby Land	Nov	Juveniles < 50 mg	80.6±0.33		21
				50–100 mg	81.7±0.29		30
				100–200 mg	81.4±0.29		14
Torres et al. (1994)	60 °C, constant mass	NW Weddell Sea	March	30–53 mm	73.3±0.71		23
		S Scotia Sea	Winter	20–53 mm	77.3±0.60		32

Mineral Ash

Dried, homogenised krill were placed in a muffle furnace at room temperature, the temperature elevated to 550 °C over half an hour, then held at this temperature for 12 hours (Postel et al., 2000; Kirkwood, 2001). Samples were cooled to room temperature over a period of one hour and transferred to a desiccator prior to weighing. Mineral ash content was expressed as the mass remaining after combustion as a percentage of original dry mass. The combustion temperature was high enough to ensure complete ignition of organic matter, but not so high as to allow a significant loss of potassium (Grove et al., 1961 in Clarke (1976)).

The majority of previous studies on krill have used 500 °C as the combustion temperature (Table 3.2). However when a trial of subsamples from the same homogenised krill tissue were ashed at both temperatures, there was found to be a higher ash content in the samples combusted at 550 °C than 500 °C. When the mineral ash obtained from combustion at 550 °C was tested for carbon content, only traces were found (a mean of 0.24 mg carbon 100 mg mineral ash $^{-1}$), suggesting complete combustion of organic carbon at this temperature and minimal carbonate from the exoskeleton.

To express mineral ash content as a percentage of frozen mass, the pre- and post ashing masses were used to calculate g ash per g dry mass. The mass of the whole dried krill, pre-homogenisation was known and so g mineral ash per whole dried krill was calculated, then this figure expressed as a percentage of the known frozen mass for each krill.

Table 3.2. Summary of mineral ash content data for *Euphausia superba* available in the literature. Measurements on whole animals unless otherwise stated. (SE, standard error; *n* = total number of krill analysed). Where there is no SE, figures in brackets show range for values observed).

Study	Analysis method	Antarctic location	Time of year	Krill analysed	% Fresh mass Mean ± SE	% Dry mass Mean ± SE	*n*
Raymont et al. (1971)	Muffle furnace at 550 °C	Peninsula	Dec	Mostly juvenile		17.0 (13.4–20.2)	20
			Jan			13.0 (12.2–13.8)	7
Clarke (pers. comm.)	Ignition at 500 °C for 12 hours	Palmer Station	Dec	Male	3.47±0.05		9
			Winter	Male	2.87±0.05		14
				Adult	3.00±0.04		15
Bykov (1975)	—	—	—	All krill	2.3–4.0		—
				<35 mm	2.6–3.1		—
				35-55 mm	2.7–3.1		—
Il'ichev (1965)	—	—	Jan	—	3.5		—
			March		3.2		—
Clarke (1980)	Ignition at 500 °C for 12 hours	South Georgia	Feb	Juvenile	3.12±0.04		—
				Male	4.13±0.37		—
				Gravid females	2.82±0.26		—
Nicol et al. (1992a)	500 °C	67°46'S 75°24'E	Feb	Laboratory maintained		9.41 (8.08–10.11)	180
				Wild sample		12.74	50
Zhu and Wang (1989)	500 °C for 12 hours. Muscle tissue only	King George Island	Jan	Male		15.07	—
				Female		16.13	—
Ferguson & Raymont (1974)	500 °C, constant mass	Bransfield Strait	Dec	Mostly juvenile		26.6 (17.4–36.9)	5
		W South Orkney Is	Jan			11.1 (10.2–12.6)	5
		E South Orkney Is	Jan			15.1 (13.1–19.1)	5
		South Georgia	Jan			13.0 (12.1–15.2)	5
			Feb			11.3 (7.8–15.1)	5
Suzuki and Shibata (1990)	—	—	Dec–Feb	All krill	3.01		—

Study	Analysis method	Antarctic location	Time of year	Krill analysed	% Fresh mass Mean ± SE	% Dry mass Mean ± SE	*n*
Sidhu et al. (1970)	A.O.A.C method	South of Tasman Sea	31 May	Juvenile		10.6	—
		North of the Ross Sea	6 April	Juvenile		9.0	—
Mauchline (1980a)	Synopsis of all available data	—	—	—	2.3–3.7		—
Ikeda and Kirkwood (1989)	Muffle furnace at 480 °C for 5 hours	Off Enderby Land	Nov	Juvenile (< 50 mg)		16.8±0.20	21
				Juvenile (50–100 mg)		18.0±0.17	29
				Adult (100–200 mg)		17.3±0.27	14
Torres et al. (1994)	500 °C for 3 hours	NW Weddell Sea	March	30–53 mm		16.1±0.65	23
		S Scotia Sea	Winter	20–53 mm		12.6±0.30	32

Protein

Protein is the most frequently determined proximate component of organic tissue, across a wide range of disciplines and applications. This range and frequency of use has resulted in the development of numerous techniques for the quantification of protein. Crossman et al. (2000) showed that the estimation of protein content can be affected by methodology and sample composition, with different analysis techniques giving markedly different results. It is therefore important to ensure an appropriate methodology is chosen to ensure the precision and accuracy of results. Previous studies of protein content in krill have applied a variety of techniques (see Table 3.3) including approximation based on nitrogen content and the spectrophotometric techniques of Lowry (1951) and Bradford. The Folin phenol method developed by Lowry (1951) is the most frequently used technique, both in studies on krill and in the wider scientific literature, principally due to its simplicity, precision and sensitivity (Peterson, 1983; Zaia et al., 2000). A number of authors have undertaken comparisons of methods of protein determination; with Crossman et al. (2000), Lucarini and Kilikian (1999) and Peterson (1983) showing the Lowry method to be one of the most accurate and reliable methods of protein determination, less subject to variation with different proteins and widely applicable.

In this study, the method of Lowry et al. (1951) as modified by Hartree (1972) was used for determining protein, following the modifications for use on krill employed by Clarke (1980). Here, the nitrogen in the protein's peptide bonds reacts with copper ions and is reduced by the addition of an acid reagent, giving a blue colour which is quantified spectrophotometrically.

Individual, frozen krill were cut into three and digested for 12 hours in 0.1M sodium hydroxide (NaOH) at 50 °C in conical flasks sealed with Parafilm to prevent evaporation. Digest volumes were standardised as 1 ml NaOH per 12 mg krill (frozen

mass). Digests were cooled and triplicate subsamples of 100 µl taken whilst agitated on a whirlmixer. A standard solution of bovine serum albumin (BSA) (Sigma-Aldrich A4503) was used at a known concentration of approximately 0.25 mg BSA ml^{-1}. A range of standard concentrations and blanks were prepared and krill digest subsamples were diluted to 1 ml total volume with water. Three chemical reagents were used in the assay:

Solute A. 2 g potassium sodium tartrate ($KNaC_4H_4O_6.4H_2O$) and 100 g sodium carbonate (Na_2CO_3) dissolved in 500 ml 1M sodium hydroxide (NaOH) and diluted with water to 1 litre.

Solute B. 2 g potassium sodium tartrate and 1 g copper sulphate pentahydrate ($CuSO_4.5H_2O$) dissolved in 90 ml water and made up to 100 ml with 1M sodium hydroxide (NaOH).

Solute C. 1 volume Folin and Ciocalteu's phenol reagent (Sigma-Aldrich F9252) diluted with 15 volumes of water.

To all samples and standards 0.9 ml of solution A was added, mixed and incubated for 10 minutes at 50 °C in a water bath. After cooling to room temperature, 0.1 ml solution B was added, tubes were mixed and incubated for 10 minutes at room temperature. Three ml of solution C was rapidly added to each tube, mixed within 1 second and incubated for 10 minutes at 50 °C. The absorbance of samples and standards was read at 650 nm on a spectrophotometer and sample absorbencies compared with those from the calibration to quantify protein concentration (see Figure 3.2 for an example of a typical calibration curve).

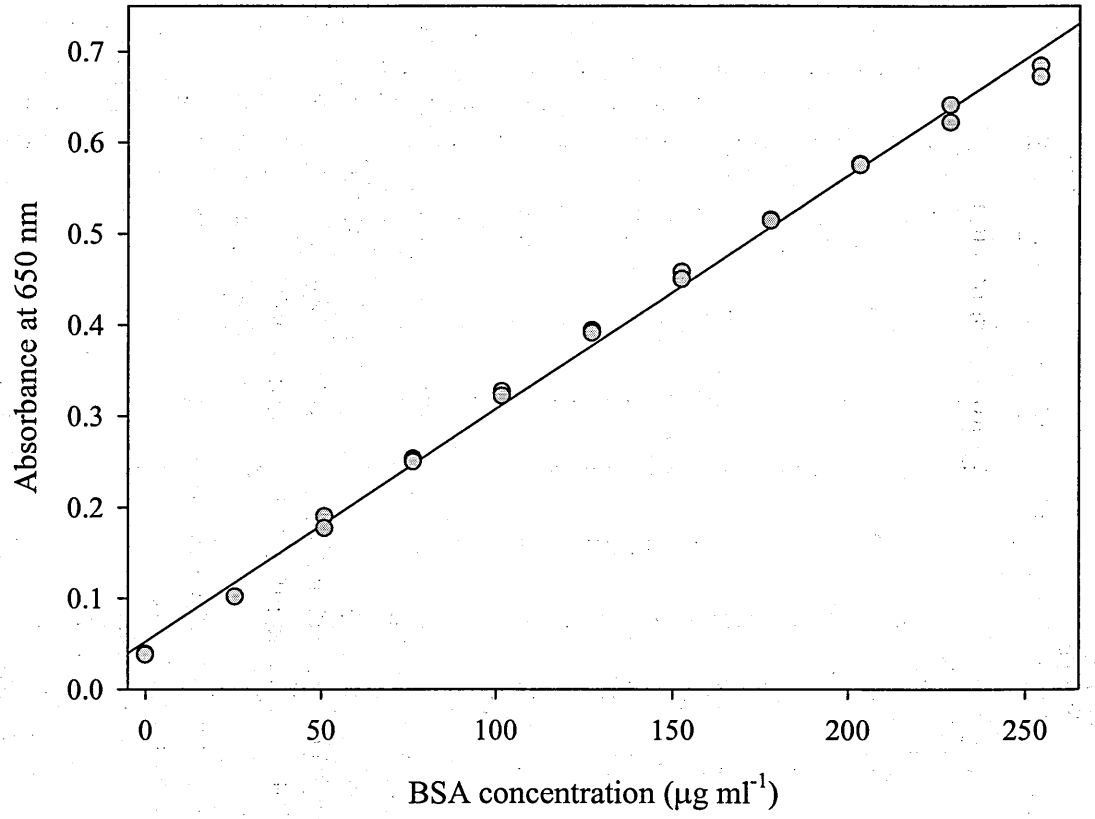

Figure 3.2. An example of a typical calibration curve for protein determination, using bovine serum albumin as a standard $R^2 = 99.6\%$.

Although BSA is the most commonly used standard in the spectrophotometric determination of proteins (Zamer et al., 1989), there are some limitations of using BSA as a known standard for quantitative determination of protein in krill, as it is not a 'krill protein'. It has been shown that BSA does not always accurately represent the protein in other marine phyla, for example Zamer et al. (1989) found that BSA underestimates protein in cnidarian tissue. By using protein isolated from anemones instead of BSA, the estimation of total body composition by CHN stoichiometric methods was improved by 15% However, BSA has been used as a standard in studies on krill by the majority of previous authors (for example Ikeda & Hing Fay, 1981; Amsler & George, 1985; Nicol et al., 1992a) and is advocated by Peterson (1983) for use as the calibration protein in his recommendations for a standardised protocol for protein determination.

Careful inspection (and residual analysis in MINITAB) shows that the calibration curve is not perfectly linear. Peterson (1983) gives a power function which best describes the fit for calibrations up to 0.7 nm maximum observed absorbance. This function was fitted to the calibration data; with the results being very similar to those obtained using the least squares fitted line, so the latter was used in line with other studies (as per Figure 3.2).

Table 3.3. Summary of protein content data for *Euphausia superba* available in the literature. Measurements on whole animals unless otherwise stated. (SE, standard error; *n* = total number of krill analysed). Where there is no SE, figures in brackets show range for values observed).

Study	Analysis method	Antarctic location	Time of year	Krill analysed	% Fresh mass Mean ± SE	% Dry mass Mean ± SE	*n*
Raymont et al. (1971)	Biuret method	Peninsula	Dec	Mostly		52.7 (41.3–61.1)	20
			Jan	juvenile		50.3 (39.8–60.6)	8
Clarke (pers. comm.)	Lowry et al. (1951) as modified by Hartree (1972)	Palmer Station	Dec	Male	10.36±0.28		6
			Winter	Male	9.40±0.47		15
				Female	11.03±0.42		9
Bykov (1975)	N x 6.25 (Assumed all nitrogenous substances are protein)	—	—	All krill	11.3–17.7		—
				<35 mm	12.8–15.0		—
				35-55 mm	14.5–16.3		—
Il'ichev (1965)	—	—	Jan	—	13.7		—
			March		17.8		—
Clarke (1980)	Lowry et al. (1951) as modified by Hartree (1972)	South Georgia	Feb	Juvenile	10.31±0.24		—
				Male	10.41±0.28		—
				Gravid females	10.63±0.32		—
Cullen et al. (2003)	Bicinchoninic acid assay (Smith et al., 1985) Abdominal muscle tissue	Deception Island	Nov '99	Male	11.11±0.37		7
				Female	9.90±0.48		10
			Feb '00	Male	10.07±0.27		13
				Female	9.81±0.31		13
			May '00	Male	12.12±0.31		10
				Female	13.02±0.37		8
			Nov '00	Male	10.95±0.37		7
				Female	9.69±1.06		10
Mauchline (1980a)	Synopsis of all available data	—	—	—	9.9–17.7		—

Study	Analysis method	Antarctic location	Time of year	Krill analysed	% Fresh mass Mean ± SE	% Dry mass Mean ± SE	*n*
Nicol et al. (1992a)	BioRad kit (based on Bradford assay)	67°46'S 75°24'E	Feb	Laboratory maintained		36.02 (31.16–42.47)	180
				Wild sample		37.50	50
Zhu and Wang (1989)	Kjeldah's N determination. Muscle tissue	King George Island	Jan	Male		66.97	—
				Female		65.67	—
Ikeda and Hing Fay (1981)	Lowry et al. (1951) BSA standard	Indian Ocean sector	Dec–Jan	Juvenile		43.3	1
Ferguson and Raymont (1974)	Biuret method	Bransfield Strait	Dec	Mostly		48.5 (41.7–58.7)	10
		W South Orkney Is.	Jan	juveniles		56.3 (50.9–66.6)	10
		E South Orkney Is.	Jan			45.7 (37.8–63.7)	10
		Nr South Georgia	Jan			48.2 (42.8–54.5)	10
			Feb			37.0 (26.6–41.6)	23
Nicol et al. (2000a)	Synopsis of all available data	—	—	—		60–65	—
Sidhu et al. (1970)	Total nitrogen x 6.25	South of Tasman Sea	31 May	Juvenile		48.9	—
		North of the Ross Sea	6 April	Juvenile		49.0	—
Suzuki and Shibata (1990)	—	—	Dec–Feb	All krill	13.6		—
Torres et al. (1994)	Lowry et al. (1951)	NW Weddell Sea	March	30–53 mm	10.6±0.21		23
		S Scotia Sea	Winter	20–53 mm	8.9±0.14		32
Atkinson et al. (2002)	Lowry et al. (1951)	SW Lazarev Sea	April	Juvenile		35±0.74 (28–45)	34
				Adult		32±0.42 (24–38)	21
Roschke (1978)	Total nitrogen x 6.25. Muscle tissue	—	April	Juvenile		58.72 (52.02–62.47)	11
			Dec–May			58.62 (49.79–64.35)	107

Carbohydrate

Determination of carbohydrates was undertaken using a method based on the colorimetric test of Dubois et al. (1956), modified for analysis of krill (Clarke, 1980). The protocol of Dubois et al. (1956) has been widely used in studies of carbohydrate content, both in studies on krill (see Table 3.4) and the wider literature. After several trials of the above method, further modifications were made to obtain the ideal digest volume and dilution and the following protocol followed:

Krill were placed in a conical flask with 10% trichloroacetic acid (TCA) (Cl_3CCO_2H) to give a concentration of 0.15 mg carbohydrate ml^{-1} TCA (based on an assumed concentration of 0.5% carbohydrate wet mass per krill – between the values for male and female krill published by Clarke (1980), see Table 3.4.) up to a maximum volume of 25 ml TCA. Flasks were incubated in a water bath for 30 minutes at 100 °C with a watch glass placed on top to prevent evaporation, then cooled and diluted with water if necessary to achieve a concentration of approximately 0.15 mg carbohydrate ml^{-1}. Triplicate 3 ml aliquots were removed from the digest with a 5 ml Gilson pipette with the tip cut off, whilst agitated on a whirlimixer, then centrifuged at 4 °C (6000 rpm, 10 minutes). Standards were prepared using a standard solution of 100 µg ml^{-1} glucose (D-glucose, Fisher G/0500). Triplicate aliquots of 0.1 ml were taken from each digest subsample after centrifugation and standards and samples made up to 1 ml with 5% TCA. To each tube (samples and standards), 1 ml of 5% phenol solution was added, followed by the quick addition of 5ml of concentrated sulphuric acid (H_2SO_4). Tubes were left to stand for 10 minutes, then vortex mixed and placed in a water bath for 20 minutes at 30 °C. Once cooled, absorbencies were read at 480 nm on a spectrophotometer and the krill digest sub samples compared with the standards to obtain carbohydrate concentrations (see Figure 3.3 for an example of a typical calibration cure).

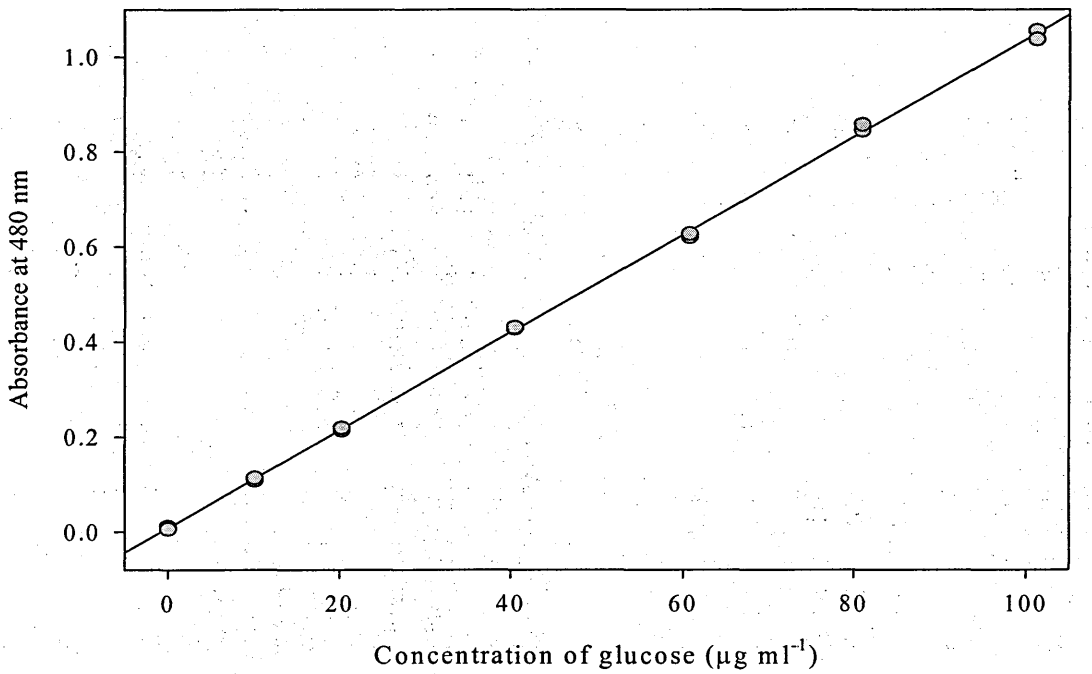

Figure 3.3. An example of a calibration curve for carbohydrate determination with D-glucose use as a standard. $R^2 = 100\%$. Duplicate samples are presented at each concentration.

Scanning absorbance readings were taken for both the krill digest and glucose standard solutions. The peak absorbance of 70 µg ml $^{-1}$ glucose solution fell at 486/487 nm, compared with a peak of 481 nm in all krill solutions. Dubois et al. (1956) suggests reading absorbencies at 490 nm for hexoses, 480 nm for pentoses and uronic acids. This agrees with the finding that glucose had a maximal absorbance closer to 490 nm, however, the composition of krill carbohydrate in terms of balance of hexoses and pentoses was unknown, so the decision was taken to use 480 nm as this was closer to the peak absorbance of the krill solution being investigated. Perhaps it would have been better to use a pentose standard when determining carbohydrate content in krill, as the absorbance peak of krill digest close to 480 suggests a pentose dominated composition, although the errors caused by this slight variation in wavelength are very small.

Although chitin is a complex polysaccharide, the assay protocol used here does not measure chitin either in its complex form, or as denatured products.

Table 3.4. Summary of carbohydrate content data for *Euphausia superba* available in the literature. Measurements on whole animals unless otherwise stated. (SE, standard error; *n* = total number of krill analysed). Where there is no SE, figures in brackets show range for values observed).

Study	Analysis method	Antarctic location	Time of year	Krill analysed	% Fresh mass Mean ± SE	% Dry mass Mean ± SE	*n*
Raymont et al. (1971)	Dubois et al. (1956)	Peninsula	Dec	Mostly		4.7 (3.6–7.1)	20
			Jan	juvenile		3.1 (2.8–3.6)	8
Clarke (pers. comm.)	Dubois et al. (1956)	Palmer Station	Dec	Male	0.57±0.05		6
			Winter	Male	0.42±0.07		7
				Female	0.42±0.10		8
Bykov (1975)	—	—	—	All krill	2.0–6.0		—
Clarke (1980)	Dubois et al. (1956)	South Georgia	Feb	Male	0.34±0.01		—
				Gravid females	0.64±0.04		—
Zhu and Wang (1989)	Dubois et al. (1956). Muscle tissue only	King George Island	Jan	Male		1.86	—
				Female		1.57	—
Mauchline (1980a)	Synopsis				0.84		—
Ferguson and Raymont (1974)	Dubois et al. (1956)	Bransfield Strait	Dec	Mostly		3.1 (2.3–3.9)	10
		W South Orkney Is.	Jan	juvenile		4.1 (3.5–4.7)	10
		E South Orkney Is.	Jan			2.1 (1.3–2.4)	10
		Nr South Georgia	Jan			2.1 (1.8–2.4)	10
			Feb			2.1 (1.3–2.6)	23
Suzuki and Shibata (1990)	Gluicide method	—	Dec–Feb	Whole krill	0.61		—
Torres et al. (1994)	Dubois et al. (1956), glucose standard	NW Weddell Sea	March	30–53 mm	0.2±0.0		23
		S Scotia Sea	Winter	20–53 mm	0.1±0.0		32
Atkinson et al. (2002)	Holland and Gabbot (1971)	SW Lazarev Sea	April	Juvenile		< 1%	34
				Adult		< 1%	21

Lipid

Lipid is by far the most studied proximate component in krill, and there is a vast body of literature dedicated to the investigation of krill lipids. Most of this work focuses on the detailed composition of these lipids, and as such involves complex procedures for analysis. However, the first stages of this process involving the extraction and quantification of total lipid are common to many (see Table 3.5); the majority using the similar methods of Folch et al. (1957) or Bligh and Dyer (1959).

Lipids were extracted following an adaptation of the method of Folch et al. (1957). Individual frozen krill were cut into three and extracted overnight at 4 °C in 25 ml chloroform : methanol (2 : 1 v/v) mixture. Samples were then homogenised, the homogeniser washed with 5 ml chloroform : methanol and the sample and wash filtered through pre-washed filter paper (Whatman number one). To the filtrate, 7.5 ml of 0.88% (w/v) potassium chloride (KCl) solution was added and the samples centrifuged (1500 rpm, 2 minutes, 4 °C). The upper layer of the spun samples containing water and methanol was discarded and the remaining chloroform removed from the extracted lipid under a constant stream of nitrogen on an N-EVAP system, until the sample was dry. The lipid was redissolved in chloroform and transferred to pre-weighed vial, with several washes to ensure complete transfer. The chloroform was then removed under nitrogen on the N-EVAP system and the samples desiccated overnight in a vacuum desiccator. Total lipid was determined gravimetrically by weighing the desiccated samples in sealed, pre-weighed vials to ±0.01 mg.

Table 3.5. Summary of lipid content data for *Euphausia superba* available in the literature. Measurements on whole animals unless otherwise stated. (SE, standard error; *n* = total number of krill analysed; * no gravid females; † median). Where there is no SE, figures in brackets show range for values observed).

Study	Analysis method	Antarctic location	Time of year	Krill analysed	% Fresh mass Mean ± SE	% Dry mass Mean ± SE	*n*
Hagen et al. (2001)	Folch et al. (1957)	Peninsula	Oct–Nov	Juvenile		10.0±0.51	10
				Sub-adult		8.6±0.37	14
				Adult*		7.0±0.21	11
			Summer	Juvenile		9.8±1.84	2
				Sub-adult		15.4±1.80	10
				Female		9.6±0.95	7
				Male		7.1±0.74	6
		Lazarev Sea	Winter	Juvenile		16.2±1.4	4
				Female		20.1	1
				Male		28.3±1.27	2
			Oct–Nov	Female		18.9±0.94	20
				Male		20.6±0.6	9
		Weddell Sea	Oct–Nov	Juvenile		10.5±1.83	13
				Sub-adult		8.9	1
				Female		10.4±0.65	46
				Male		11.0±0.88	16
			Summer	Juvenile		27.7±0.58	5
				Female		30.9±2.05	11
				Male		24.3±3.55	6
			Autumn	Juvenile		42.9±0.55	4
				Sub-adult		40.4±0.75	12
				Female		37.9±1.12	27
				Male		39.4±1.41	22

Study	Analysis method	Antarctic location	Time of year	Krill analysed	% Fresh mass Mean ± SE	% Dry mass Mean ± SE	*n*
Raymont et al. (1971)	Folch et al. (1957)	Peninsula	Dec	Mostly		13.3 (6.4–27.8)	26
			Jan	juvenile		27.1 (23.3–32.9)	8
Bykov (1975)	—	—	—	All krill	1.1–5.8		—
				<35 mm	2.5–4.1		—
				35–55 mm	4.1–5.8		—
Clarke (1980; 1984)	Bligh and Dyer (1959)	South Georgia	21 Jan	Juvenile	4.09±0.39		11
		Bransfield Straight	29 Jan	Female	5.72±0.57		9
		South Georgia	3 Feb	Male	2.41±0.48		8
				Female	5.23±0.24		8
			8 Feb	Female	6.01±0.46		3
			13 Feb	Female	5.63±0.23		6
			21 Feb	Female	5.30±0.33		13
			1 March	Juvenile	6.49±2.78		2
				Gravid females	6.33±0.49		20
				Male	5.33±1.04		12
Nicol et al. (1992a)	Modified Bligh and Dyer (1959)	67°46'S 75°24'E	Feb	Laboratory maintained		23.73 (19.5–27.1)	180
				Wild sample		22.46	50
Zhu and Wang (1989)	Soxhlet extraction with ether. Muscle tissue	King George Island	Jan	Male		11.03	
				Female		12.00	
Mauchline (1980a)	Synopsis	—	—	—	1.1-7.0		—
Tsuyuki and Itoh (1976)	—	Enderby Land	Dec–Feb	—	3.4		—
Hagen et al. (1996)	Dichloromethane:methanol extracted, analysed by Folch et al. (1957)	SE Weddell Sea	Spring	All krill		10.5±0.45	71
			Summer	25–56 mm		28.2±1.44	23
			Autumn			39.2±0.70	65

Study	Analysis method	Antarctic location	Time of year	Krill analysed	% Fresh mass Mean ± SE	% Dry mass Mean ± SE	*n*
Clarke (pers. comm.)	—	Palmer Station	Dec	Male	1.37±0.12		6
			Winter	Male	4.32±0.37		14
				Female	3.46±0.21		25
Ferguson and Raymont (1974)	Folch et al. (1957). Rehydrated individuals	Bransfield Strait	Dec	Mostly juveniles		16.1 (14.0–20.2)	8
		W South Orkney Is	Jan			19.1 (12.9–24.6)	8
		E South Orkney Is	Jan			31.1 (22.7–43.2)	10
		Nr South Georgia	Jan			27.6 (20.3–40.0)	8
			Feb			34.3 (20.0–43.1)	22
Nicol et al. (2000a)	Synopsis	—	—	—		10-20	—
Suzuki and Shibata (1990)	—	—	Dec–Feb	All krill	1.84		—
Kryuchkova and Makarov (1969b)	—	—	19 Feb	Juvenile	4.9	20.4	—
				Female	2.8	13.4	—
			2 March	Juvenile	4.4	17.7	—
				Male	1.6	6.8	—
				Female	4.2	17.1	—
			11 March	Juvenile	7.2	27.4	—
				Male	1.8	8.7	—
				Female	4.3	17.2	—
Torres et al. (1994)	Bligh and Dyer (1959) extraction, analysed with charring method of Marsh and Weinstein (1966)	NW Weddell Sea	March	30–53 mm	3.6±0.50		23
		S Scotia Sea	Winter	20–53 mm	4.0±0.27		32
Sidhu et al. (1970)	—	S Tasman Sea	May	—	6.1		—
		Ross Sea	April		8.0		—
Atkinson et al. (2002)	Hagen et al. (2001)	SW Lazarev Sea	April	Juvenile		36±0.89 (26–44)	34
				Adult		44±1.77 (30–58)	21

Study	Analysis method	Antarctic location	Time of year	Krill analysed	% Fresh mass Mean ± SE	% Dry mass Mean ± SE	*n*
Virtue et al. (1993)	Modified Bligh and Dyer (1959)	Prydz Bay	Summer	Juvenile	2–21		20
				Sub-adult	8–15		20
				Male	1–3		50
				Gravid female	19–30		20
				Spent female	12–17		20
Mayzaud et al. (1998b)	Bligh and Dyer (1959), either gravimetric or Barnes and Blackstock (1973) determination	Indian sector (60–64 S, 30–50 E)	Feb	Sub-adult	2.9±0.16 (1.1–4.7)		33
				Male	2.2±0.12 (0.7–5.0)		54
				Gravid females	3.3±0.21 (1.6–4.8)		14
				Spent females	2.8±0.27 (1.1–4.8)		20
Shibata (1983)	—	103–114 °E, 63–65 °S	27 Dec	41–50 mm	1.11		—
			30 Dec		1.33		—
			4 Jan		1.65		—
			12 Jan		1.92		—
			26 Jan		2.73		—
			30 Jan	< 24 mm	2.90		—
				25–40 mm	3.56		—
				41–50 mm	2.72		—
				Gravid females (>51 mm)	4.32		—
			25 Feb	41–50 mm	3.95		—
Pond et al. (1995)	Chloroform : methanol Folch et al. (1957)	South Georgia	Jan–Feb	Juvenile	4.16† (0.95–12.5)		76
				Male	1.81† (0.72–8.20)		48
				Female*	3.48† (1.18–9.40)		41

Study	Analysis method	Antarctic location	Time of year	Krill analysed	% Fresh mass Mean ± SE	% Dry mass Mean ± SE	*n*
Mayzaud et al. (1998a)	Bligh and Dyer (1959)	Indian sector (60–80 °E, 50–67 °S)	Feb	Juvenile		11.40±1.73	5
				Male		11.45±1.28	5
				Gravid females		17.63±1.98	5
Sidhu et al. (1970)	Smith et al. (as cited in Sidhu et al., 1970)	South of Tasman Sea	31 May	Juvenile		21.9	—
		North of the Ross Sea	6 April	Juvenile		27.4	—
Ju and Harvey (2004)	Chloroform:methanol, summation of major lipid classes detected by flame ionisation	Marguerite Bay	July–Aug	Sub-adult (30–40 mm)		17.5 (15–30)	10
				Adult (45–62 mm)		20.5 (15–30)	17
Il'ichev (1965)	Quick method	—	Dec	—	0.84		—
			Jan	—	1.2		—
				Male	1.1–1.3		
				Female	2.2–2.5		
			Feb	—	1.2		—
			March	—	3.4–7.7		—

Nucleic acids

Despite the application of total nucleic acid content, and ribonucleic acid to deoxyribonucleic acid (RNA : DNA) ratios, acids as measures either of nutritional condition (Wagner et al., 1998) or as growth rate indicators (Dagg & Littlepage, 1972; Båmstedt & Skjoldal, 1980; Wagner et al., 2001) in zooplankton, very little work has been done to date on proximate measures of nucleic acids in *E. superba* (Ikeda, 1989; Cullen et al., 2003). As such, we were keen to try and obtain direct chemical estimates of both RNA and DNA content in this study.

Nucleic acid assays are employed in a wide range of biochemical and physiological studies, and as such many methods exist for the determination of RNA and DNA. Measurements of nucleic acid concentrations were first carried out using spectrophotometric methods, but have been increasingly replaced by spectrofluorimetric techniques which are both more specific and more sensitive (Grémare & Vétion, 1994) and require less tissue for analysis. The sequential enzymatic determination method described by Bentle et al. (1981) has been widely used in studies on fish, especially larvae (Westerman & Holt, 1988) and in recent years adopted for studies of nucleic acids in copepods (Wagner et al., 1998; Geiger et al., 2000; Gorokhova & Kyle, 2002) and krill (Cullen et al., 2003). It was thought, therefore, that this would provide a current methodology that has previously produced good results with marine zooplankton.

The enzymatic determination method of Bentle et al. (1981) measures the change in fluorescence of an ethidium bromide-complexed polynucleotide substrate after the sequential addition of enzymes (DNase then RNase) to break down nucleic acids. Many different modifications of this technique exist (Clemmesen, 1993), and a long period of experimentation was necessary to develop a satisfactory protocol for krill tissue. However, in the end I was unable to produce accurate and precise values for the RNA and DNA

content of krill using this technique. I therefore attempted the determination of nucleic acids by spectrophotometric methods using the colorimetric methods of Mejbaum (1939) for RNA and Burton (1956) for DNA, together with the dual absorbance method described in Munro and Fleck (1969) and the results from the two different methodologies were compared.

Samples were prepared in the same way prior to both analyses following Fraser (1998): Whole, pre-weighed krill were homogenised in 0.2M perchloric acid (PCA) at a concentration of 100 mg frozen tissue ml^{-1}; then the homogeniser washed with an equal volume of PCA which was combined with the homogenate. Triplicate 2 ml aliquots were removed from the homogenate with a 5 ml Gilson pipette with the tip cut off, whilst agitated on a whirlimixer. Subsamples were centrifuged at 4 °C (6000 rpm, 10 minutes) to separate the supernatant from the precipitated protein, RNA and DNA. The supernatant was discarded and the pellet was washed twice in 0.2M PCA (4 °C, 6000 rpm, 10 minutes).

The pellet was resuspended by vortex mixing with 4.5 ml distilled water plus 0.5 ml 3M sodium hydroxide (NaOH), and then incubated at 37 °C for 1 hour. After incubation, 1.1 ml of the solution was removed for potential protein determination. The solubilised protein pellet was re-precipitated and total RNA released by the addition of 0.9 ml 20% PCA. The samples were centrifuged, as above, and the supernatant saved for RNA determination.

The remaining pellet, containing precipitated DNA, was washed with 10 ml 2% PCA and centrifuged (as previously), with the supernatant discarded to remove any trapped RNA. Five ml of 0.6N PCA was added to the pellet and incubated at 70 °C for 30 minutes in a water bath. After incubation, the samples were spun again and the supernatant containing the DNA saved for analysis.

Colorimetric assays:

The content of RNA in the supernatant was determined using the Orcinol method of Mejbaum (1939). The samples were compared against known RNA (Type IV calf liver, Sigma R-7250) standard concentrations, with 1 ml of sample or standard combined with an equal volume of 0.5M hydrochloric acid (HCl). Two ml of Orcinol reagent (iron chloride ($FeCl_3$) dissolved in concentrated hydrochloric acid (HCl) at a concentration of 0.2 mg ml^{-1} with 120 mg Orcinol added per 20 ml $FeCl_3$/HCl solution) was added to the samples and standards and heated at 110 °C in a heating block for 35 minutes. The absorbance of the samples and standards were read at 665 nm on a spectrophotometer, once cool. The absorbance of krill supernatant samples was compared with the standards to obtain RNA concentrations (see Figure 3.4 for an example of a typical calibration cure).

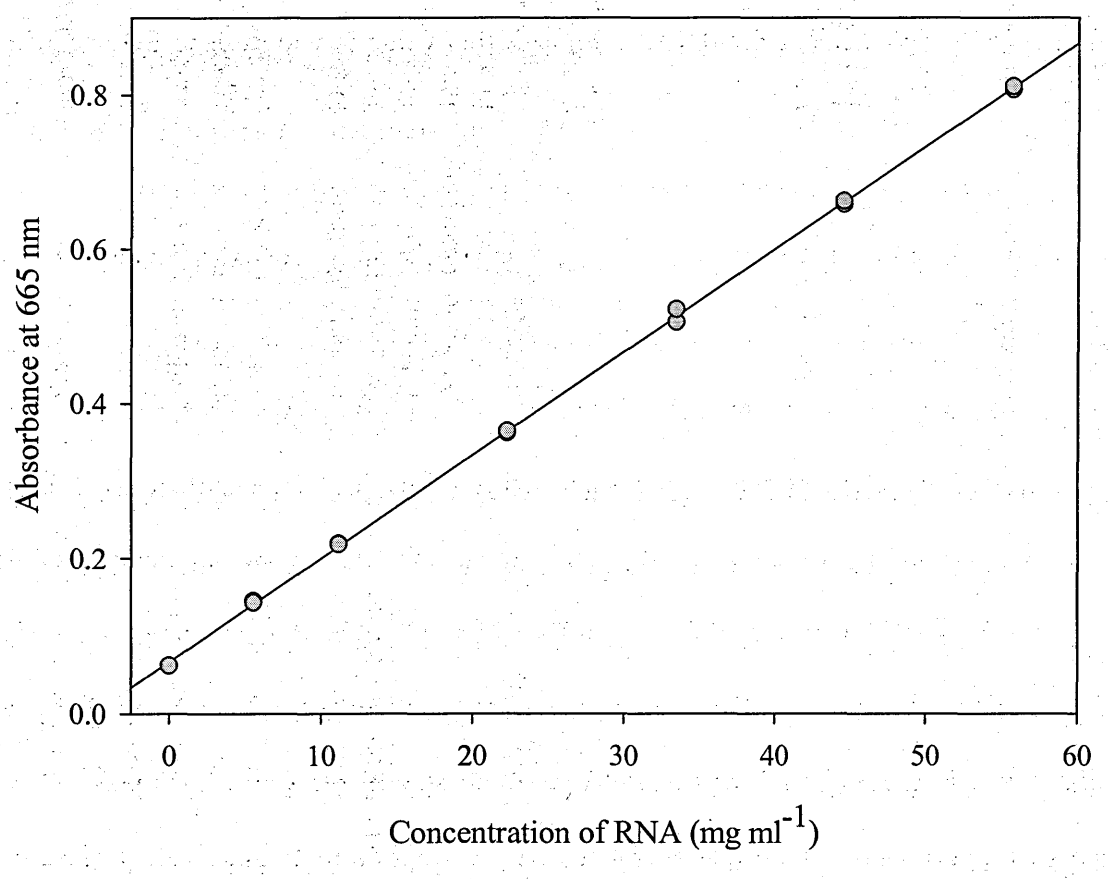

Figure 3.4. An example of a typical calibration curve for RNA determination using Type IV calf liver RNA as a standard. $R^2 = 100\%$.

Samples and standards were scanned over a range of absorbancies, with the peak absorbance found to be at 668 nm across a range of RNA concentrations. However, when the concentrations of RNA determined by reading at 668 nm were compared with those obtained by scanning at 665 nm, they were not found to be statistically different (*t*-test, $p>0.05$) and so the published absorbance of 665 nm was used for consistency with other studies.

The RNA standard as supplied is a crude extract and the purity cannot be guaranteed by the manufacturer (Sigma), so the concentration of the standard stock was determined by reading its absorbance at 260 nm against a blank of 0.5 ml 0.5M sodium hydroxide (NaOH) and 0.5 ml 1M hydrochloric acid (HCl), mixed and diluted to 10 ml with 0.5M hydrochloric acid (HCl). Traditionally, it is said that at 260 nm the spectrophotometric equivalent of one absorbance unit is 40 µg RNA, calculated from the specific absorption coefficient for RNA. Although this value is widely reported and used in the literature, the original source of this value of 40 µg RNA could not be found. However, under the above conditions, one absorbance unit at 260 nm has been calculated to be equivalent to 32 µg RNA (Fraser, pers. com, Houlihan et al., 1995). Table 3.7 shows a comparison of the results obtained from this method using different methods of determining the concentration of the RNA standard. The purity of the RNA standard was also investigated by elemental analysis; subsamples of each RNA standard used were analysed for carbon, hydrogen and nitrogen content with an elemental analyser (CE Instruments EA 1108) using cycloheanone-2,4-dinitro-phenylhydrazone as a standard. Based on the assumption that all nitrogen in the crude extract supplied commercially is attributable to RNA, standard 1 was 88.03% pure and standard 2 was 72.7% pure. This compares with purities of 68.8% and 56.7% with a spectrophotometric equivalent of 32 µg RNA and 86.0% and 70.9% for an equivalent of 40 µg RNA for standards one and two respectively when compared with known mass.

Table 3.6. Elemental composition of RNA standards compared to pure RNA (assumed equal proportions of each base). Two replicate samples from each standard analysed (SE, standard error).

	Carbon		Hydrogen		Nitrogen	
	Mean	SE	Mean	SE	Mean	SE
Standard 1	30.1	0.2	4.4	0.3	14.4	0.6
Standard 2	29.8	0.2	4.3	0.1	11.9	0.3
Pure RNA	35.6		3.4		16.4	

It was attempted to determine the DNA content of the supernatant preparation using the colorimetric assay of Burton (1956), which uses diphenylamine as the coloured indicator to quantify the amount of DNA in solution. However, because of the low concentrations of DNA found in krill, I was not able to quantify the concentration of DNA in the krill supernatant with a high enough degree of accuracy or precision using this method, despite several modifications.

Dual absorbance method:

An alternate spectrophotometric method for determining the nucleic acid content of prepared samples is the dual absorbance method. This method is used widely (for example Bauer et al., 1989; Glasel, 1995; Nicklas & Buel, 2003), partly because of its simplicity, although as the method was developed on rat muscle tissue it is potentially inappropriate to apply it without modification to other animal tissue. The method of Munro and Fleck (1969) was used, with the modification of Ashford and Pain (1986) applied to the calculation of RNA concentration.

The absorbance of the RNA and DNA supernatant preparations was read at 260 and 232 nm on a spectrophotometer. Concentration of RNA was calculated as:

Equation 3.1

$$\text{RNA } (\mu g\ ml^{-1}) = (32.9 \times \text{Absorbance}_{260}) - (6.11 \times \text{Absorbance}_{232})$$

DNA concentration was calculated with the following formulae:

Equation 3.2

$$\text{DNA } (\mu g\ ml^{-1}) = 10.53 \times [(3.17 \times \text{Absorbance}_{260}) - (0.75 \times \text{Absorbance}_{232})]$$

RNA concentrations determined through the dual absorbance method were compared with those obtained using the orcinol colorimetric technique (see Table 3.7).

Table 3.7. Comparison of the nucleic acid content (% frozen mass) of Antarctic krill. determined by the various methods described in the text. (SE, standard error; CV, coefficient of variation; *n* = total number of krill analysed).

	Mean	SE	Range	CV (%)	*n*
RNA (mass)	0.4805	0.0207	0.2526–0.9016	29.906	48
RNA (absorbance x32)	0.2879	0.0138	0.1584–0.6139	33.137	48
RNA (absorbance x40)	0.3599	0.0172	0.1979–0.7674	33.148	48
RNA (dual absorbance)	0.2538	0.0110	0.1218–0.4250	27.778	41
DNA (dual absorbance)	0.0977	0.0038	0.04643–0.16164	26.586	48

On the basis of this detailed comparative study, it was decided to use the RNA concentrations determined using the orcinol colorimetric method, with the concentration of the standard calculated using the spectrophotometric equivalent of one absorbance unit at 665 nm equalling 32 μg RNA. The orcinol method provided more accurate results (the dual absorbance technique failed on several occasions to determine RNA concentration), with a similar level of precision. Also, this technique has been specifically modified for use on other marine animal tissue, including invertebrates, by previous authors (Houlihan et al., 1995; Fraser et al., 2001; Fraser et al., 2002),. The spectrophotometric equivalent of 32 μg RNA was chosen as this is the value used by the authors of this modified method, and it compares well with the results derived using the dual absorbance method.

Table 3.8. Summary of RNA content data for *Euphausia superba* available in the literature (% fresh mass). Measurements on whole animals unless otherwise stated. (SE, standard error; *n* = total number of krill analysed; * original data presented as μg RNA g muscle^{-1}; † original data presented as % of protein (Protein value of 11.97% fresh krill used for conversion, data from this study)). Where there is no SE, figures in brackets show range for values observed).

Study	Analysis method	Antarctic location	Time of yr.	Krill analysed	Mean ± SE	*n*
Cullen et al. (2003)	Sequential enzymatic determination on muscle tissue (Bentle et al., 1981)*	Deception Island	Nov 1999	Male	0.00053±0.000045	9
				Female	0.00062±0.000066	11
			Feb 2000	Male	0.00047±0.000051	10
				Female	0.00069±0.000156	10
			May 2000	Male	0.00028±0.000015	14
				Female	0.00022±0.000044	10
			Nov 2000	Male	0.00073±0.000044	10
				Female	0.00056±0.000059	9
Ikeda (1989)	Absorption at 260 nm (Dagg & Littlepage, 1972)†	Off Wilkes Land	Jan–Feb	All krill	0.611 (0.299–0.982)	41

Table 3.9. Summary of DNA content data for *Euphausia superba* available in the literature (% fresh mass). Measurements on whole animals unless otherwise stated. (SE, standard error; *n* = total number of krill analysed; * original data presented as μg DNA g muscle^{-1}).

Study	Analysis method	Antarctic location	Time of yr.	Krill analysed	Mean ± SE	*n*
Cullen et al. (2003)	Sequential enzymatic determination on muscle tissue (Bentle et al., 1981)*	Deception Island	Nov 1999	Male	0.00063±0.000078	9
				Female	0.00043±0.000043	11
			Feb 2000	Male	0.00022±0.000018	10
				Female	0.00019±0.000023	10
			May 2000	Male	0.00017±0.000015	14
				Female	0.00014±0.000010	15
			Nov 2000	Male	0.00027±0.000015	10
				Female	0.00017±0.000023	9

Chitin

Chitin has previously been determined in marine invertebrates by several methods; gravimetric determination of material remaining after alkaline digestion (Ferguson & Raymont, 1974; Clarke, 1980), by the colorimetric determination method of Tsuji et al. (1969) as used by Torres et al. (1994), enzymatic determination (Jeuniaux, 1965) used in a range of studies (Voss-Foucart et al., 1995; Ravaux et al., 1998; Cauclie et al., 1999; Ravaux et al., 2000) and High Performance Liquid Chromatography (HPLC) analysis for acetic acid (Nicol et al., 1992a) or glucosamine (Espie & Roff, 1995). No single method dominates the literature for chitin studies in krill, as all have inherent advantages and disadvantages (as discussed in Nicol and Hosie (1993)). Figure 3.5 and Table 3.12 present the range of values obtained for krill using these differing methodologies.

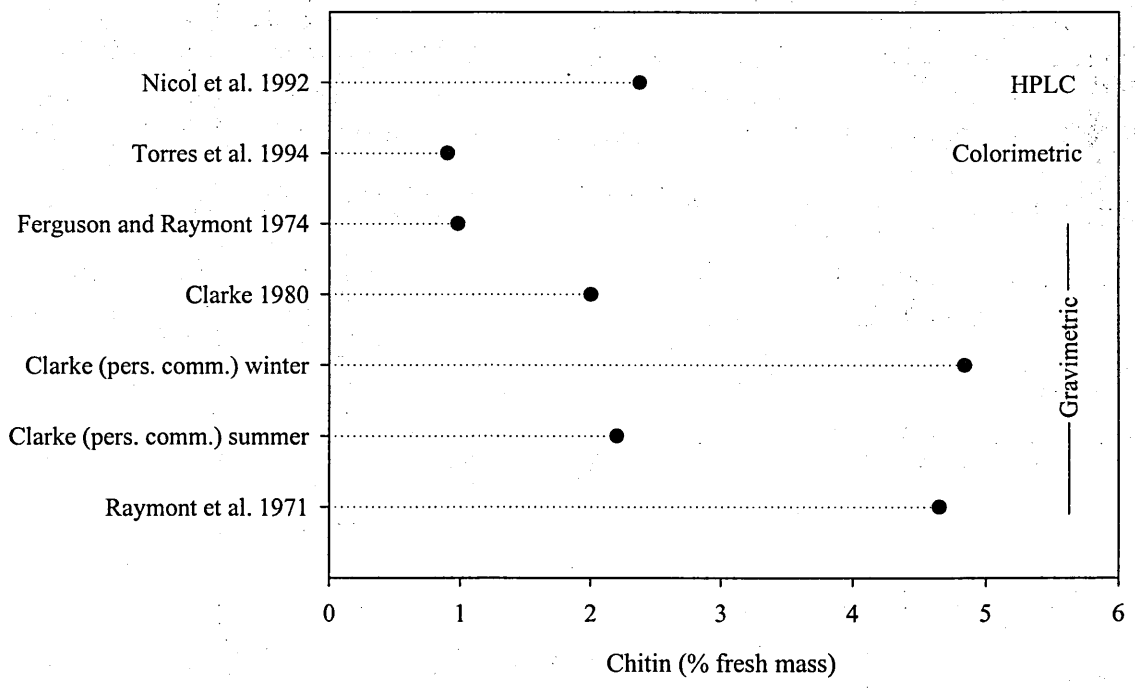

Figure 3.5. Comparison of mean values for chitin content in Antarctic krill from the literature using a range of different techniques. For studies with more than one value for a season, or where the sexes are separated, the mean value of these data has been presented here.

It was decided to use the gravimetric method for the analysis in this study, as HPLC methods involved expensive equipment and the colorimetric method was complex, time

consuming and yield the lowest value of the range of available data, where as the gravimetric technique was simple and there was a range of existing data with which to compare results. Also, the gravimetric assay allowed for subsequent checks of purity of the chitin.

Frozen individual krill were cut into thirds and digested for 12 hours in 1M sodium hydroxide (NaOH) at 50 °C in conical flasks sealed with Parafilm©. After digestion, samples and blanks (NaOH only) were filtered through pre-weighed and pre-washed (with dilute HCl followed by distilled water) Whatman glass microfiber filter papers (GF/C). The filtrate was rinsed with three washings of 1M hydrochloric acid (HCl) followed by three washings of distilled water. Filter papers were then dried in an oven at 60 °C for 24 hours and re-weighed. Blank filters were weighed and a mean blank value was subtracted from each final mass of the krill samples. It was assumed that everything retained on the filter paper was chitin – precipitate and exoskeleton 'ghost'; however the exoskeleton was also removed from the filter paper and weighed separately. In addition, these exoskeleton 'ghosts' were retained and C, H, N and mineral ash content determined on a homogenised, pooled sample.

There was a significant difference between the percentage chitin values obtained by the two methods; with the mean for the first method (entire filtrate) being higher (3.05% wet mass) and more variable (CV = 51.2%) than the results gained from measuring the 'ghost' only (mean 1.19%, CV = 15.7%). However, when the elemental analysis results for these 'ghosts' were considered; it was shown that they were not pure chitin (see Table 3.10).

Table 3.10. Elemental analysis of krill 'ghosts' remaining after chitin analysis (digestion in NaOH). Elemental analysis on homogenised pooled sample of 10 'ghosts', 10 replicate subsamples. Ash content determined from remainder of sample. All values % dry mass. Pure chitin values calculated from chemical composition of n-acetyl-glucoseamine by mass.

	Mean (± SE)	Pure chitin
Mineral ash	5.99	—
Carbon	54.2±0.18	43.6
Hydrogen	7.87±0.097	6.41
Nitrogen	4.12±0.046	6.36

The C, H, N and mineral ash analysis of the 'ghosts' indicated that the exoskeleton is rich in chitin, but not pure chitin. If it is assumed that all the nitrogen in the 'ghost' is attributable to chitin (6.36% nitrogen by mass), it could be hypothesised that the ghosts contained 64.8% chitin. However, it is likely that the precipitate contained some chitin not present in the exoskeleton (from the gut and chitin being laid down in preparation for a new moult) and potentially some chitin that had separated from the exoskeleton during the process of digestion. For these reasons, it was decided to use the chitin values obtained from weighing the entire filtrate, in preference to those from the exoskeleton 'ghost' only. These data were corrected with the assumption (from the CHN data) that 'chitin' determined in this method is 64.8% pure after subtraction of mineral ash content of the exoskeletons (5.99%) (see Table 3.10), to calculate chitin value used in this study. Table 3.11 presents a summary of the results obtained using the different methods for calculating total chitin.

Table 3.11. Summary of results for differing methods of chitin determination. All data expressed as percentage frozen mass. See text for explanation of methodology. (SE, standard error; CV, coefficient of variation; *n* = total number of krill analysed).

Method of chitin determination	Mean	SE	Range	CV (%)	*n*
Mass of entire filtrate	3.05	0.228	0.40–6.79	51.2	47
Mass of 'ghost'	1.19	0.028	0.78–1.64	15.7	46
Mass of 'ghost' adjusted for purity	0.73	0.017	0.48–1.00	15.7	46
Filtrate mass adjusted for purity	1.86	0.139	0.24–4.13	51.2	47

Table 3.12. Summary of chitin content data for *Euphausia superba* available in the literature. Measurements on whole animals unless otherwise stated. (SE, standard error; *n* = total number of krill analysed). Where there is no SE, figures in brackets show range for values observed).

Study	Analysis method	Antarctic location	Time of year	Krill analysed	Fresh mass Mean ± SE	Dry mass Mean ± SE	*n*
Raymont et al. (1971)	Digested in KOH Raymont et al. (1969)	Peninsula	Dec	Mostly	4.1 (2.5–6.1)		16
			Jan	juvenile	5.2 (4.1–6.1)		3
Clarke (pers. comm.)	Digested in NaOH	Palmer Station	Dec	Male	2.20±0.38		9
			Winter	Male	4.84±1.15		5
Clarke (1980)	Digested in NaOH	South Georgia	Feb	Male	2.11±0.21		—
				Gravid females	1.90±0.23		—
Nicol et al. (1992a)	Orthophosphoric (H_3PO_4) acid digestion, HPLC analysis for acetic acid	67°46'S 75°24'E	Feb	Laboratory maintained	2.7		180
				Wild sample	2.04		50
Mauchline (1980a)	Synopsis	—	—	—	0.23–2.45		—
Ferguson and Raymont (1974)	Digested in NaOH, washed with chloroform : methanol	Bransfield Strait	Dec	Mostly		5.7 (2.2–10.5)	6
		W South Orkney Is.	Jan	juvenile		3.5 (2.5–4.9)	5
		E South Orkney Is.	Jan			5.5 (4.0–7.3)	5
		Nr South Georgia	Jan			4.0 (3.2–5.2)	5
			Feb			3.3 (2.2–5.1)	5
Nicol et al. (2000a)	Synopsis	—	—	—	2.4–2.7		—
Suzuki and Shibata (1990)	—	—	Dec–Feb	All krill	0.51		—
Sidhu et al. (1970)	Hackman (Sidhu et al., 1970)	South of Tasman Sea	31 May	Juvenile		2.8	—
		North of the Ross Sea	6 April	Juvenile		2.3	—
Torres et al. (1994)	Colorimetric determination of hexosamines (Tsuji et al., 1969)	NW Weddell Sea	March	30–53 mm	1.0±0.02		23
		S Scotia Sea	Winter	20–53 mm	0.8±0.02		32

Results

Water content

Overall, for both cruises combined, the mean water content was 75.8% with a range of 70.0–84.5% and a coefficient of variation (CV) of 3.75%. However, there were significant differences between the water content of the krill sampled on different years; (ANOVA, $F_{1, 224} = 49.62$, $p < 0.001$), with the krill from JR82 having a significantly greater mean water content (see Table 3.13).

Table 3.13. Water content of Antarctic krill (% frozen mass). (SE, standard error; *n* = total number of krill analysed, length is given in mm). Data in italics is shown for pooled sexual maturity classifications (adult females, sub-adult and adult males).

	JR70				JR82				All data			
	Mean	SE	*n*	Length	Mean	SE	*n*	Length	Mean	SE	*n*	Length
All	74.4	0.21	98	33–62	76.8	0.26	128	20–60	75.8	0.19	226	20–62
J	75.0	0.52	14	33–44	75.8	0.31	58	20–44	75.6	0.27	72	20–44
FS	74.4	0.47	22	35–46	72.0	—	1	42	74.3	0.46	23	35–46
FA1	—	—	—	—	78.1	0.38	33	33–54	78.1	0.38	33	33–54
FA2	—	—	—	—	76.9	0.41	3	47–52	76.9	0.41	3	47–52
FA3	73.8	0.09	2	53–55	77.8	1.74	3	49–56	76.2	1.36	5	49–56
FA4	73.3	0.25	12	51–62	74.1	0.48	13	49–58	73.7	0.29	25	49–62
FA5	—	—	—	—	81.8	0.69	10	49–60	81.8	0.69	10	49–60
FA	*73.3*	*0.22*	*14*	*51–62*	*77.8*	*0.40*	*62*	*33–60*	*77.0*	*0.38*	*76*	*33–62*
MS1	73.7	0.34	19	36–46	78.6	—	1	33	73.9	0.40	20	33–46
MS2	73.7	0.57	12	41–47	74.6	—	1	48	73.8	0.53	13	41–48
MS3	72.0	—	1	51	—	—	—	—	72.0	—	1	51
MS	*73.6*	*0.29*	*32*	*36–51*	*76.6*	*1.96*	*2*	*33–48*	*73.8*	*0.31*	*34*	*33–51*
MA1	73.3	0.45	5	54–56	77.2	0.20	2	45–47	74.4	0.78	7	45–56
MA2	77.6	0.43	11	50–61	78.1	0.37	3	48–56	77.7	0.35	14	48–61
MA	*76.3*	*0.60*	*16*	*50–61*	*77.0*	*0.31*	*5*	*45–56*	*76.6*	*0.48*	*21*	*45–61*

For JR70, the mean water content was 74.4%, ranging from 70.0 to 79.2% with a CV of 2.81%, compared with a mean of 76.8%, range of 71.5 to 84.5% and a CV of 3.76% for JR82. When krill from JR82 caught from stations falling within the same area sampled during JR70 (n=7, six J and one FA1) are considered in isolation, the mean is 73.9% (SE 0.84) (Juveniles; mean 74.3%, SE 0.88), which compares well with krill from JR70 (see Table 3.13). Where there are sufficient numbers (J and FA4) a comparison between the

two cruises for individual sexual maturity stages showed no difference in the means (*t*-test, $p>0.1$).

There were clear differences with water content between the sexes, for data from both cruises pooled (ANOVA, $F_{11, 214} = 19.39$, $p < 0.001$), for JR70 krill, (ANOVA, $F_{8, 89} = 6.76$, $p < 0.001$) and JR82 krill (ANOVA, $F_{10, 117} = 10.35$, $p < 0.001$) (Table 3.13, Figure 3.6). When the sexual maturity classes were pooled, difference between the sexes were of the same magnitude in JR70 krill (ANOVA, $F_{4, 93} = 6.71$, $p < 0.001$), but reduced in the JR82 krill (ANOVA, $F_{4, 123} = 4.91$, $p = 0.001$). In JR70 krill there was a decline in water content from high levels in juveniles to lower values as the females matured. For male krill this appeared also to be the case, until the spermatophore producing MA2 stage was reached where water content is considerably greater than for any other stage. This trend was less clear in krill caught during JR82, however it could be argued that there was still a general trend in female krill for water content to decrease with maturity (although the single value for FS is low) until spawning, when water content rises significantly. The low numbers of sub-adult males sampled prevent a clear trend emerging, but mature males had higher water contents than those sampled the previous year, with the difference between mature males producing spermatophores and those not less marked.

For both cruises pooled there was no relationship between length and water content, or in either cruise separately. However, when individual sexual maturity stages are considered alone, there is a significant negative relationship between water content and length in juvenile krill from JR82 ($r^2 = 18.7\%$, $p = 0.001$), with a similar, but non significant trend observed in juveniles from JR70. For all krill there was a significant negative relationship between frozen mass and water content ($r^2 = 5.2\%$, $p = 0.001$), but when considered by cruise no such relationship was detected. Again, considering individual sexual maturity stages in isolation, a significant negative relationship emerged in JR82 juvenile krill ($r^2 = 26.6\%$, $p > 0.001$).

No difference in the water content of JR70 krill could be detected with swarm, event, shelf location or chlorophyll content of sampling station. With animals caught during JR82 however, there were significant differences between sampling events (ANOVA, $F_{23, 104} = 2.31$, $p < 0.002$), with a trend showing increasing water content with the more southerly stations. This is highlighted by a significant negative relationship between latitude ($r^2 = 9.5\%$, $p < 0.001$) and longitude ($r^2 = 8.8\%$, $p = 0.001$) and water content; with krill from higher latitudes and more westerly longitudes containing more water.

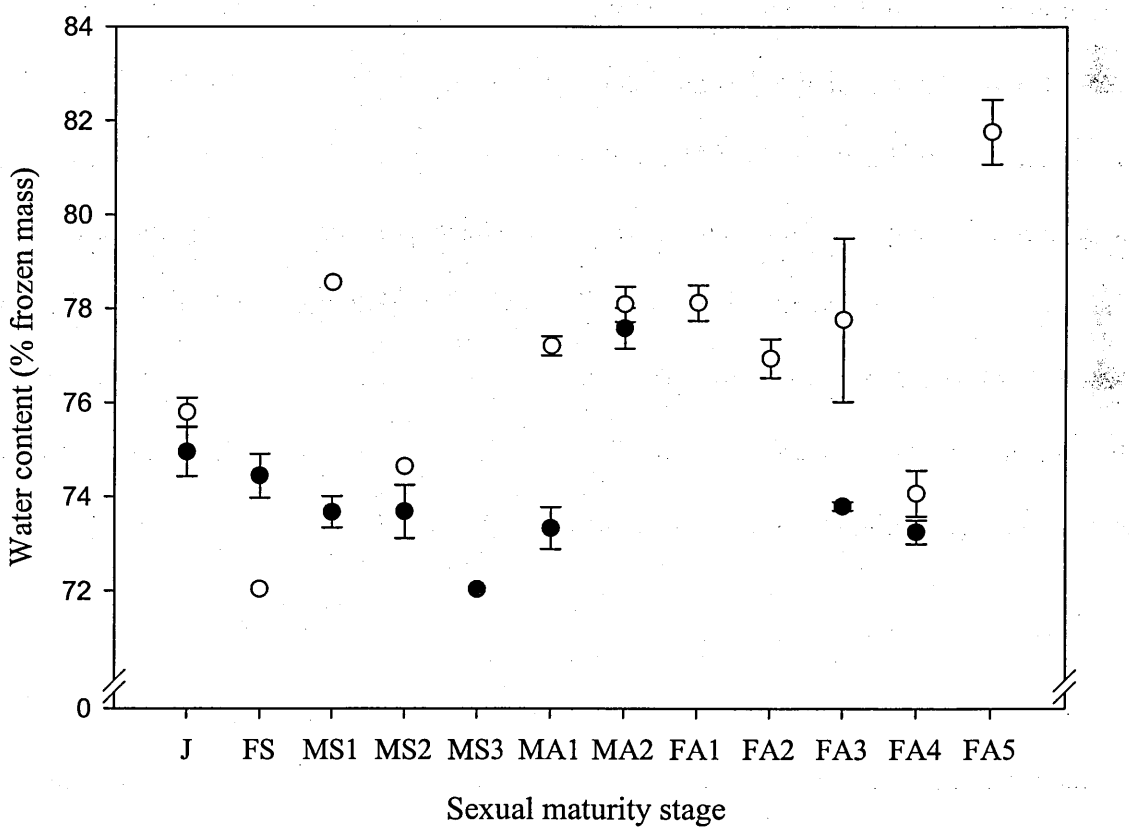

Figure 3.6. Differences in total water content (% of frozen mass) between sexual maturity stages in krill from JR70 (filled circles) and JR82 (open circles). Values are means ± 1 standard error.

There was a significant negative relationship with the chlorophyll *a* concentration of the surface water at sampling stations (see Appendix C) and krill water content for JR82 krill ($r^2 = 4.5\%$, $p = 0.019$), however, it seems this result is an artefact of an exceptionally

high chlorophyll value of 5.97 mg Chl *a* m^{-3} observed at station 649 as there was no relationship when krill sampled at this station were removed from the analysis.

Krill were caught in the ice or near the ice edge at only two stations, but the presence of ice does not seem to affect the water content of the animals or the mean Chl *a* value of sampling stations (ANOVA, $p > 0.1$). (A summary table of sampling station location, shelf position, chlorophyll *a* concentration and sea ice conditions is given in Appendix C).

Mineral Ash

Mineral ash composition of the JR70 samples ranged from 5.90% to 15.55% dry mass and 1.64% to 3.36% frozen mass. Mean frozen mass was 2.89%, with a CV of 11.54% and mean dry mass 11.47% with a CV of 17.93% (Table 3.14).

Table 3.14. Mineral ash content of Antarctic krill from JR70. (SE, standard error; n = total number of krill analysed). Data in italics is shown for pooled sexual maturity classifications (adult females, sub-adult and adult males).

	Mineral ash (% dry mass)		Mineral ash (% wet mass)			
	Mean	SE	Mean	SE	n	Length (mm)
All	11.47	0.29	2.89	0.05	49	33–61
J	12.43	0.59	3.05	0.08	7	33–42
MS1	10.93	0.24	2.88	0.05	11	38–46
MS2	10.71	0.65	2.87	0.09	4	41–47
MS3	10.03	—	2.83	—	1	51
MS	*10.82*	*0.23*	*2.88*	*0.04*	*16*	*38–51*
MA1	11.07	0.24	2.93	0.03	2	54–55
MA2	14.76	0.37	3.31	0.03	6	50–61
MA	*13.83*	*0.66*	*3.21*	*0.07*	*8*	*50–61*
FS	11.95	0.45	2.97	0.04	11	36–46
FA3	9.56	—	2.53	—	1	55
FA4	8.41	0.54	2.23	0.13	6	51–60
FA	*8.57*	*0.48*	*2.27*	*0.12*	*7*	*51–60*

For JR82 samples, mineral ash was calculated only as a percentage of dry mass; ranging from 7.794% to 24.194%, with a mean of 14.265 and a CV of 22.43%. Additional mineral ash data had been collected from JR70 from samples used for a study of growth rate (see Chapter 4) and there was found to be no significant difference between these krill and those from JR70 for which data is presented above (Table 3.14) (t-test, $t_{154} = 0.71$, $p = 0.479$). In order to facilitate a better comparison with JR82 data, these data were pooled, with a range of 5.05% to 19.84%, mean of 11.30% and a CV of 21.7% (Table 3.15).

Table 3.15. A comparison of mineral ash composition of Antarctic krill sampled from the Scotia Sea on consecutive years. (SE, standard error; *n* = total number of krill analysed). Data in italics is shown for pooled sexual maturity classifications (adult females, sub-adult and adult males).

	JR70 pooled				JR82			
	Mean	SE	*n*	Length (mm)	Mean	SE	*n*	Length (mm)
All	11.30	0.20	156	31–61	14.27	0.31	105	21–60
J	11.83	0.54	10	31–42	14.85	0.47	46	21–44
FS	11.23	0.29	50	34–46	10.95	—	1	42
FA1	11.85	—	1	49	14.26	0.30	25	38–54
FA2	13.51	—	1	unknown	13.59	0.53	3	47–52
FA3	12.20	1.70	4	53–57	14.38	1.27	3	49–56
FA4	8.46	0.40	8	51–60	9.59	0.57	12	49–58
FA5	15.91	2.05	3	53–54	18.22	1.03	8	49–60
FA	*11.15*	*0.87*	*17*	*49–60*	*13.75*	*0.46*	*51*	*38–60*
MS1	10.78	0.35	46	31–46	15.40	—	1	33
MS2	11.51	0.57	21	35–53	11.15	—	1	48
MS3	10.43	0.41	2	51–55	—	—	—	—
MS	*10.99*	*0.29*	*69*	*31–55*	*13.27*	*2.12*	*2*	*33–48*
MA1	11.07	0.24	2	54–55	14.46	0.05	2	45–47
MA2	14.71	0.32	7	50–61	15.74	0.41	3	46–56
MA	*13.90*	*0.59*	*9*	*50–61*	*15.23*	*0.39*	*5*	*45–56*

For all data pooled, there was a significant negative relationship between mineral ash (% dry mass) and length (r^2 = 2.2%, p = 0.018) and for JR82 samples alone (r^2 = 11.6%, p = 0.017), although there was no such relationship with JR70 samples (frozen or dry mass). Frozen mass data was only available for the original JR70 data, however a significant negative relationship existed between frozen mass and mineral ash (% frozen mass) (r^2 = 9.3%, p = 0.002), but not with mineral ash (% dry mass). There was a significant difference between sexual maturity stages (ANOVA, $F_{8, 40} = 11.48$, $p < 0.001$) and by pooled sex (ANOVA, $F_{4, 44} = 15.61$, $p < 0.001$) for mineral ash (% dry mass) and between sexual maturity stages (ANOVA, $F_{8, 40} = 15.98$, $p < 0.001$) and pooled sex (ANOVA, $F_{4, 44} = 26.46$, $p < 0.001$) for mineral ash (% frozen mass) for JR70 krill. The same is true for krill from JR82 for different sexual maturity stages (ANOVA, $F_{10, 64} = 6.45$, $p < 0.001$) but not by pooled sex (Table 3.15, Figure 3.7).

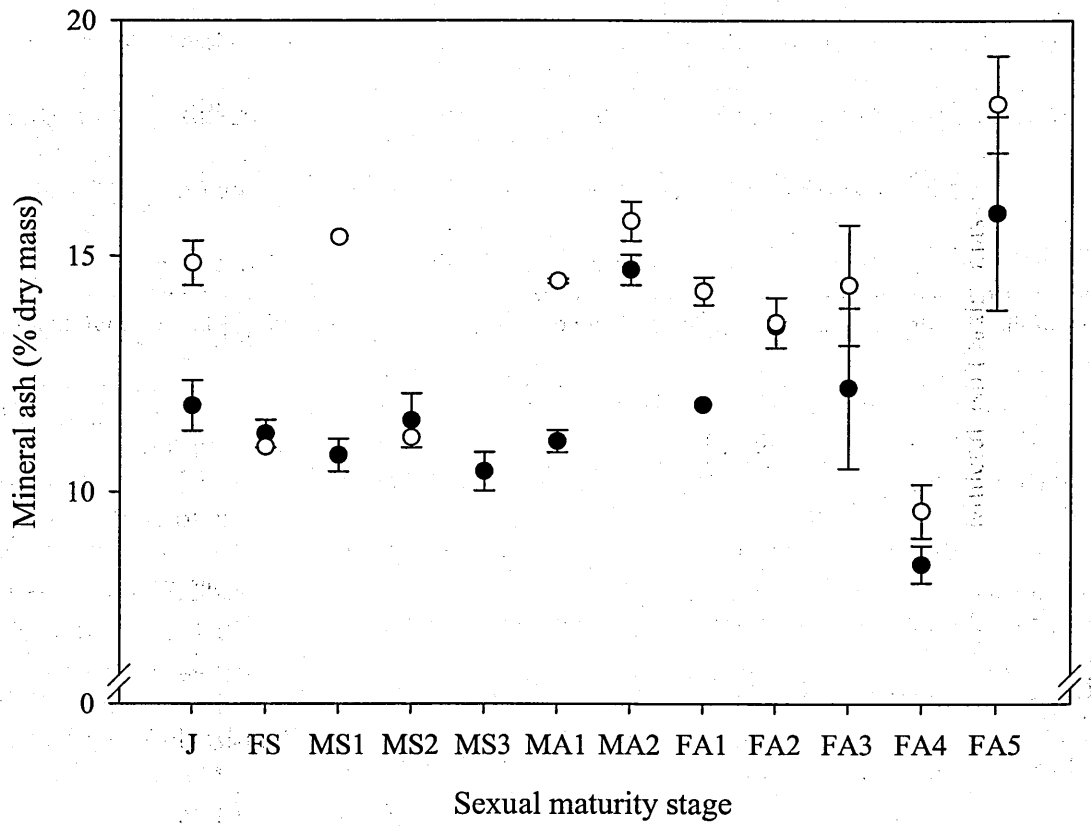

Figure 3.7. Differences in total mineral ash content (% of dry mass) between sexual maturity stages in krill from JR70 (filled circles) and JR82 (open circles). Values are means ± 1 standard error.

It can be seen that krill from both sampling seasons follow the same basic trend in mineral ash composition with different sexual maturity stages, with the outliers being attributable to single data points, except in the case of the juvenile krill.

For the pooled JR70 krill there was no significant difference between the mineral ash content of krill from different swarms or sampling locations and no relationship between mineral ash and chlorophyll *a* concentration or on- or off-shelf location. With krill from JR82 no significant relationship existed between mineral ash composition and sampling event or chlorophyll *a* concentration. A comparison between JR70, JR70 growth rate samples and JR82 samples showed a significant difference between experiments, (ANOVA, $F_{2, 258} = 35.80$, $p < 0.001$), but no difference between the two JR70 experiments (ANOVA, $F_{1, 1544} = 0.35$, $p = 0.555$), indicating that pooling of the JR70 data sets was acceptable.

Protein

The overall mean protein content was 11.97%, with a range of 10.64–13.60% and a CV of 5.81%. There was a significant difference between protein and sexual maturity stage (ANOVA, $F_{8, 34} = 2.44$, $p = 0.033$) for all sex classes individually, and for the pooled maturity stages (ANOVA, $F_{4, 38} = 3.56$, $p = 0.015$) (Table 3.16). There was no difference between sampling events, or with on- or off-shelf stations. There was no relationship between protein content and chlorophyll concentration at sampling stations.

There was a significant positive relationship between length and protein content ($r^2 = 19.8\%$, $p = 0.003$) and frozen mass and protein ($r^2 = 24.6\%$, $p = 0.001$). However, it was thought that the differences between sexual maturity stage are more likely to result in significant differences in protein content between individual krill, rather than length alone, for example MA1 and MA2 animals have approximately the same mean length, yet have significantly different protein contents; furthermore length increases from J–MS3 samples, yet protein decreases.

Within sexual maturity stages, length is more likely to be a significant determining factor for protein content – as the animal grows and increases muscle mass, the percentage of total body protein will increase. A possible explanation for the decrease in protein composition from juveniles to sub-adult stages could be that, as the animals mature, resources are put into the development of sexual characteristics and not into muscle growth. Once sexual characteristics are fully formed, energy is again available to utilise for muscle growth, as indicated by the high protein levels in mature male and female animals.

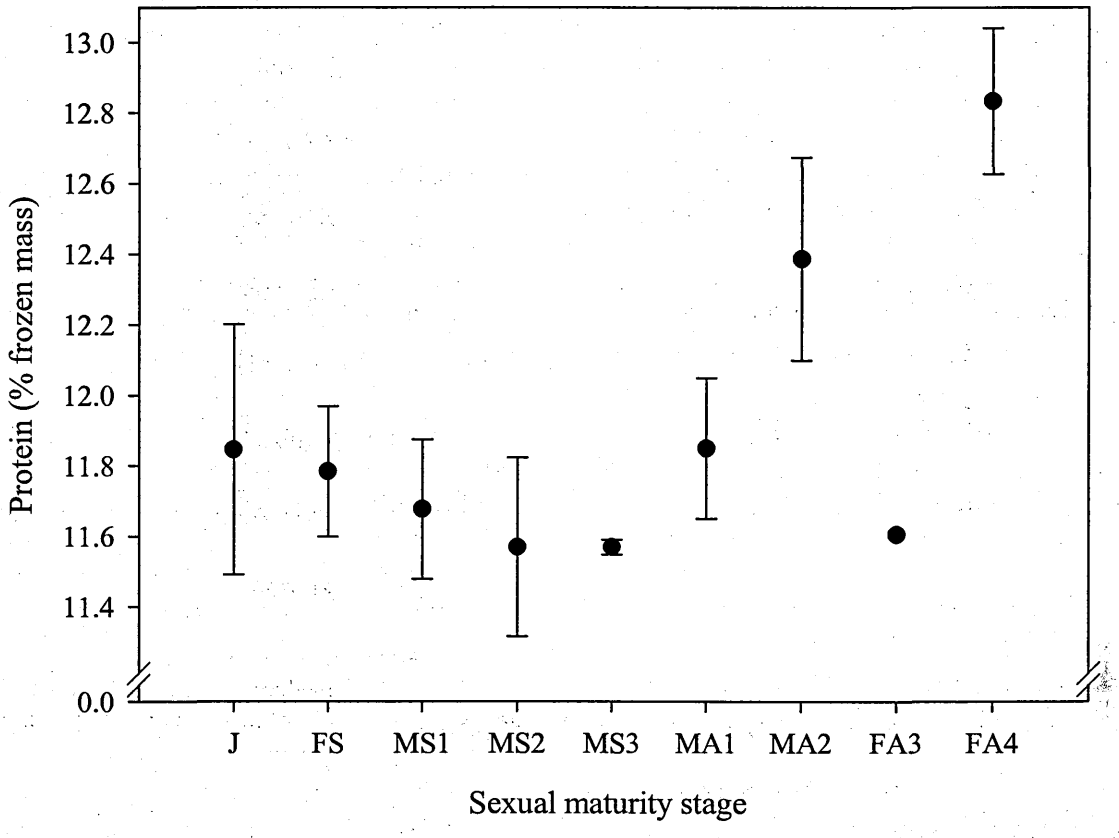

Figure 3.8. Difference in total protein content (% of frozen mass) between sexual maturity stages in krill from JR70. Values are means ± 1 standard error.

Table 3.16. Protein content of Antarctic krill. (SE, standard error; *n* = total number of krill analysed). Calculation of protein (% dry mass) involves the use of mean water content data. Data in italics is shown for pooled sexual maturity classifications (adult females, sub-adult and adult males).

	Protein (% frozen mass)				
	Mean	SE	Protein (% dry mass)	*n*	Length (mm)
All	12.0	0.11	46.7	43	33–61
J	11.9	0.36	47.3	7	33–44
MS1	11.7	0.20	44.4	6	39–45
MS2	11.6	0.25	44.0	4	40–48
MS3	11.6	0.02	41.4	2	48–50
MS	*11.6*	*0.12*	*44.1*	*12*	*39–50*
MA1	11.9	0.20	44.4	3	52–56
MA2	12.4	0.29	55.3	5	51–59
MA	*12.2*	*0.21*	*51.3*	*8*	*51–59*
FS	11.8	0.19	46.1	9	36–46
FA3	11.6	—	44.3	1	50
FA4	12.8	0.21	48.0	6	51–61
FA	*12.7*	*0.25*	*47.5*	*7*	*50–61*

Carbohydrate

When the results were analysed by experimental run (separate laboratory days), a significant difference was found, with the first run having significantly greater carbohydrate values than the other five runs; once data from this run was removed there was no significant difference between experimental runs. The source of this analytical variation was not clear, and all data presented therefore excludes the anomalous day's analysis.

The overall mean carbohydrate content was 0.39%, with a range of 0.17–0.73% and a CV of 27.2%. There was a significantly difference between carbohydrate and sexual maturity stage (ANOVA, $F_{7, 31} = 3.77$, $p = 0.005$) for all sex classes individually, and for the pooled classification (ANOVA, $F_{4, 38} = 2.90$, $p = 0.036$) (Table 3.17, Figure 3.9).

Table 3.17. Carbohydrate content of Antarctic krill. (SE, standard error; *n* = total number of krill analysed). Data in italics is shown for pooled sexual maturity classifications (adult females, sub-adult and adult males).

	Carbohydrate (% frozen mass)		Carbohydrate		
	Mean	SE	(% dry mass)	*n*	Length (mm)
All	0.39	0.02	1.54	39	34–62
J	0.37	0.03	1.48	5	34–41
MS1	0.43	0.03	1.61	6	36–46
MS2	0.20	0.03	0.75	2	46–47
MS3	0.30	0.03	1.08	3	49–54
MS	*0.35*	*0.03*	*1.33*	*11*	*36–54*
MA1	0.37	0.03	1.38	3	49–55
MA2	0.40	0.06	1.80	4	52–58
MA	*0.39*	*0.03*	*1.63*	*7*	*49–58*
FS	0.39	0.02	1.51	10	36–46
FA4	0.51	0.06	1.92	6	52–62

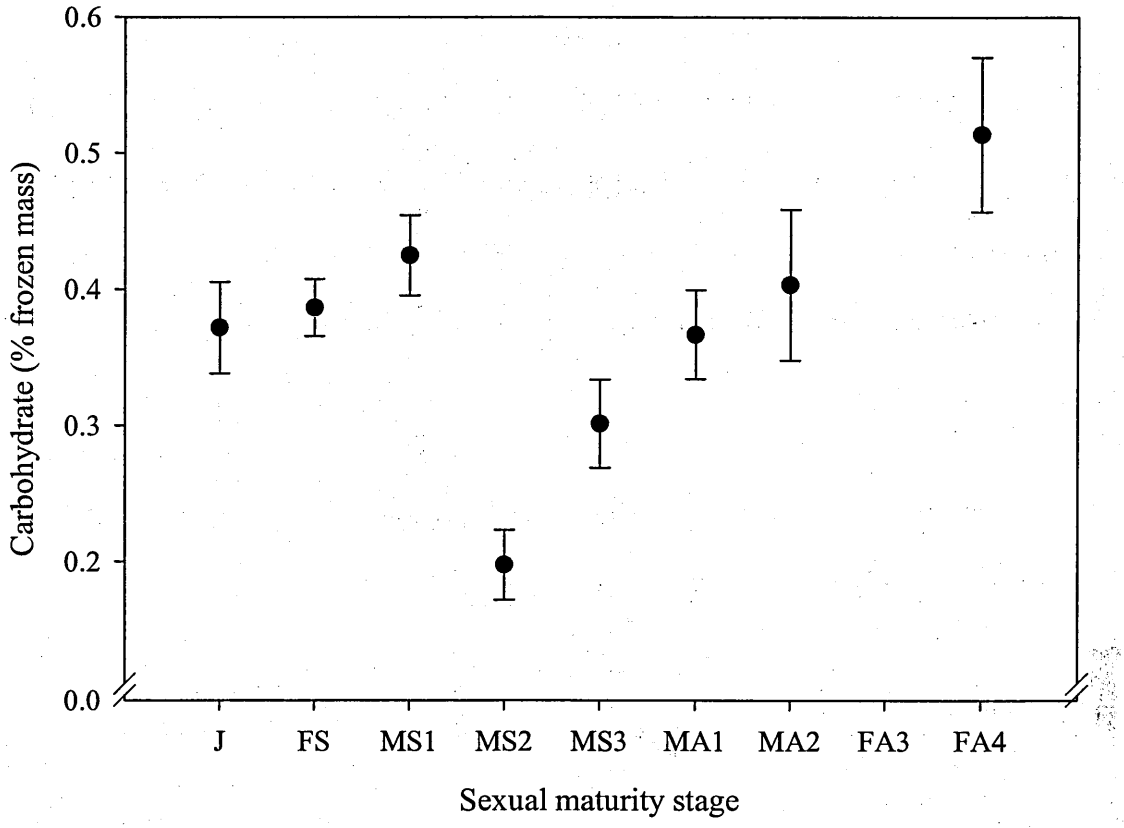

Figure 3.9. Difference in total carbohydrate content (% of frozen mass) between sexual maturity stages in krill from JR70. Values are means ± 1 standard error.

There was no relationship between length or frozen mass and carbohydrate. There was, however, a significant difference between sampling event and carbohydrate content (ANOVA, $F_{14, 24} = 2.86$, $p = 0.012$). There was a significant difference between on-shelf and off-shelf samples (ANOVA, $F_{1, 37} = 16.13$, $p < 0.001$), with off-shelf samples having a higher mean carbohydrate content. However, there was no significant difference between the chlorophyll values of on- and off-shelf stations, or no significant correlation between chlorophyll content of surface water at sampling stations and carbohydrate content of krill from those stations. This would suggest that the difference between on- and off-shelf swarms was in the sexual maturity composition, rather than as a result of environmental variability between stations. For example, there are more FA4 krill, with a significantly higher percentage carbohydrate sampled from off-shelf stations, and both MS2 krill which had significantly lower carbohydrate content were caught on-shelf.

Lipid

The overall mean lipid content was 6.42% with a range of 1.69–11.08% and a CV of 33.3%. There is a significant difference in lipid content between the sexes (Table 3.18, Figure 3.10), (ANOVA, $F_{8, 42} = 11.25$, $p < 0.001$). When the sex classes are pooled however, the difference between the sex classes is reduced (ANOVA, $F_{4, 46} = 4.30$, $p = 0.005$). There is no significant relationship between length or frozen mass and lipid content. However, when each sexual maturity stage is considered alone, significant relationships emerge, although the numbers are small. In juveniles, there is a trend for lipid to increase with length ($p = 0.049$), no trend in FS, MS2, MA2 or FA4 and a significant increase with length in MS1 ($p = 0.014$).

Table 3.18. Lipid content of Antarctic krill. (SE, standard error; *n* = total number of krill analysed). Data in italics is shown for pooled sexual maturity classifications (adult females, sub-adult and adult males).

	Lipid (% frozen mass)				
	Mean	SE	Lipid (% dry mass)	*n*	Length (mm)
All	6.42	0.30	25.1	51	34–61
J	6.16	0.71	24.6	7	34–42
MS1	6.28	0.71	23.8	8	37–47
MS2	8.10	0.21	30.7	6	42–48
MS3	7.42	0.88	26.5	2	49–50
MS	*7.10*	*0.42*	*26.9*	*16*	37–50
MA1	9.81	1.27	36.8	2	54–55
MA2	2.57	0.31	11.5	7	52–57
MA	*4.18*	*1.11*	*17.6*	*9*	*52–57*
FS	7.30	0.31	28.6	12	37–46
FA3	7.23	—	27.6	1	52
FA4	6.35	0.20	23.7	6	51–61
FA	*6.47*	*0.21*	*24.27*	*7*	*51–61*

There is not a significant difference between swarms (ANOVA, $F_{18, 32} = 2.34$, $p = 0.017$) but the difference between events (swarms pooled) is significant (ANOVA, $F_{15, 35} = 3.06$, $p = 0.003$). There was no difference between on- and off-shelf samples, although there was a difference in chlorophyll content between on- and off-shelf samples (ANOVA,

$F_{1, 49} = 5.51$, $p = 0.023$). There was no relationship between environmental chlorophyll and krill lipid content.

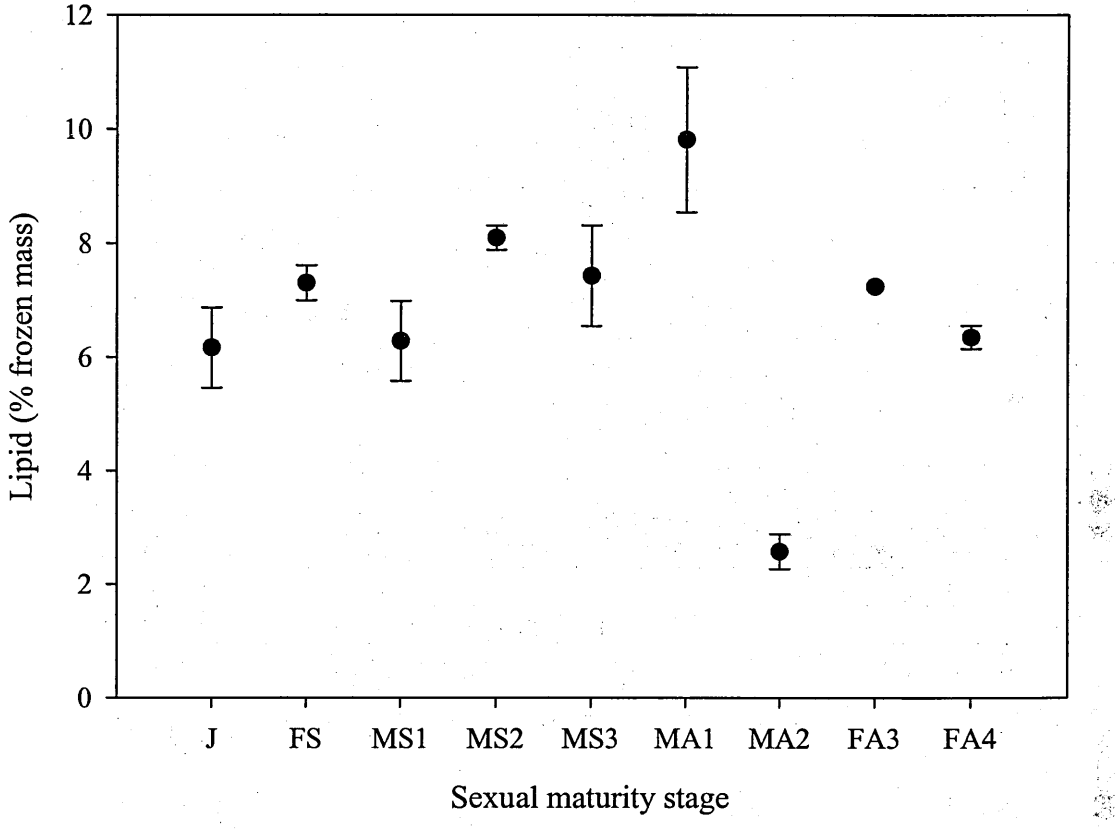

Figure 3.10. Differences in total lipid content (% of frozen mass) between sexual maturity stages in krill from JR70. Values are means ± 1 standard error.

Nucleic acids

The overall mean RNA content was 0.29% with a range of 0.16–0.61% and a CV of 33.1%. There was a significantly difference between RNA and sexual maturity stage (ANOVA, $F_{8, 39} = 5.92$, $p < 0.001$) for all sex classes individually, and for the pooled classification (ANOVA, $F_{4, 43} = 9.06$, $p < 0.001$) (Table 3.19, Figure 3.11). The mean DNA content was 0.10% with a range of 0.05–0.16% and a CV of 26.6%. There was no significant difference between DNA and sexual maturity stage, however, there was a small significant difference when all sex classes are pooled (ANOVA, $F_{4, 43} = 2.61$, $p < 0.049$) (Table 3.19, Figure 3.12).

Table 3.19. Nucleic acid content of Antarctic krill. (SE, standard error; *n* = total number of krill analysed). Data in italics is shown for pooled sexual maturity classifications (adult females, sub-adult and adult males).

	RNA (% frozen mass)		DNA (% frozen mass)				
	Mean	SE	Mean	SE	RNA : DNA	*n*	Length (mm)
All	0.29	0.01	0.10	0.00	3.04	48	35–61
J	0.28	0.02	0.11	0.01	2.61	7	35–43
MS1	0.33	0.02	0.11	0.01	2.94	6	35–42
MS2	0.23	0.02	0.09	0.01	2.51	7	43–49
MS3	0.27	0.02	0.09	0.01	3.16	2	50–51
MS	*0.28*	*0.02*	*0.10*	*0.01*	2.77	*15*	*35–51*
MA1	0.20	0.03	0.08	0.01	2.35	3	54–56
MA2	0.20	0.02	0.09	0.01	2.35	5	52–57
MA	*0.20*	*0.01*	*0.09*	*0.00*	2.35	*8*	*52–57*
FS	0.29	0.02	0.17	0.01	2.73	11	34–46
FA3	0.45	—	0.11	—	4.53	1	55
FA4	0.42	0.06	0.08	0.01	5.50	6	53–61
FA	*0.42*	*0.05*	*0.08*	*0.01*	5.36	*7*	*53–61*

When RNA : DNA ratios were considered, a significant difference with sex was shown (ANOVA, $F_{8, 39} = 28.55$, $p < 0.001$), although this is attributable to the gravid females (FA3 and FA4) only, as the other sexual maturity stages show no difference in nucleic acid ratios (see Figure 3.13). Similarly, the same is true when considering length (Figure 3.13) and frozen mass in relation to nucleic acid ratios; the significant positive

relationship was entirely attributable to elevated values for gravid female krill compared to all other maturity stages.

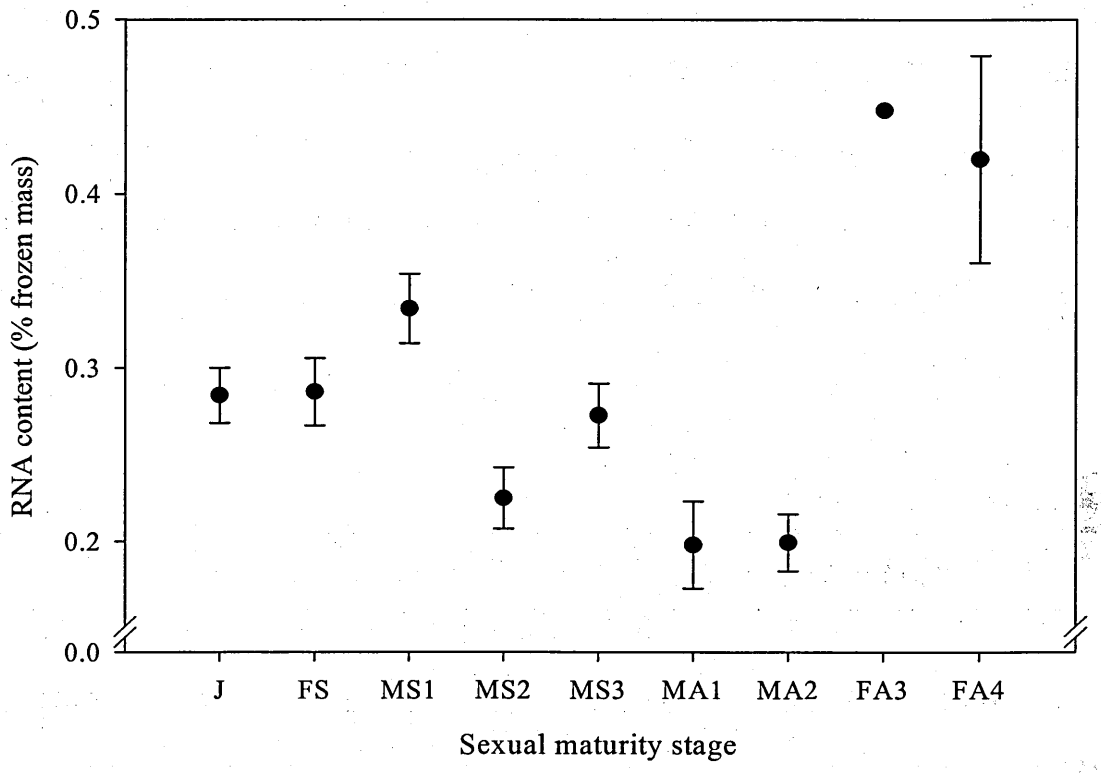

Figure 3.11. Differences in total RNA content (% of frozen mass) between sexual maturity stages in Antarctic krill. Values are means ± 1 standard error.

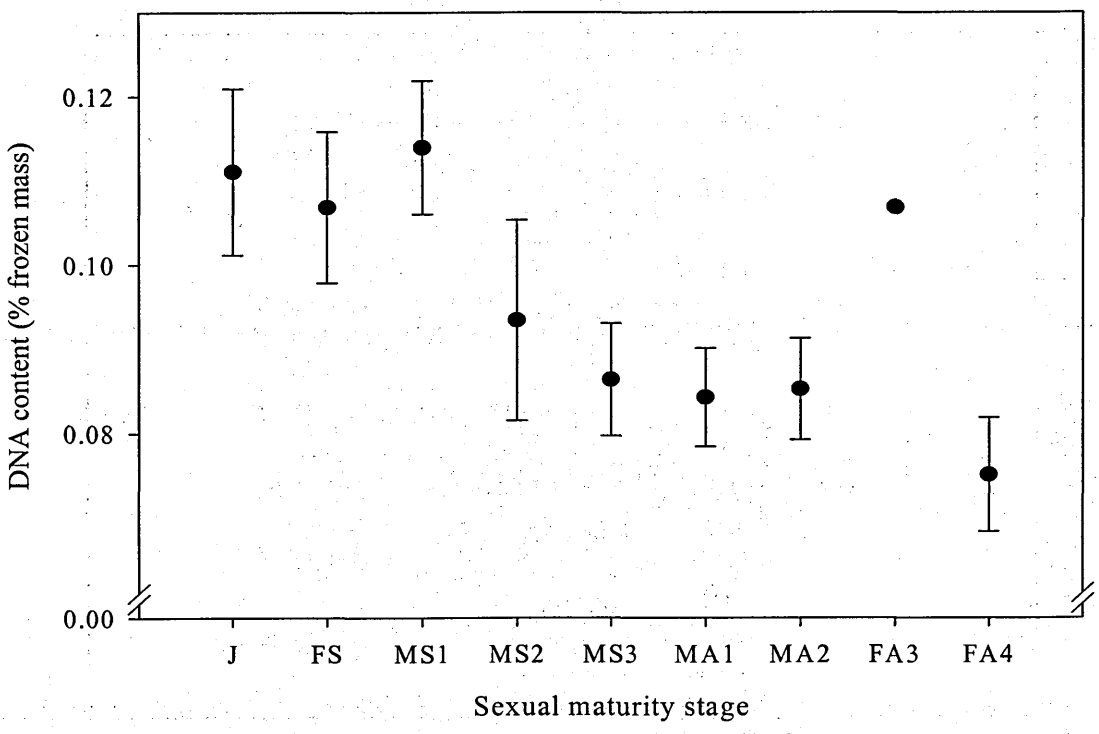

Figure 3.12. Differences in total DNA content (% of frozen mass) between sexual maturity stages in Antarctic krill. Values are means ± 1 standard error.

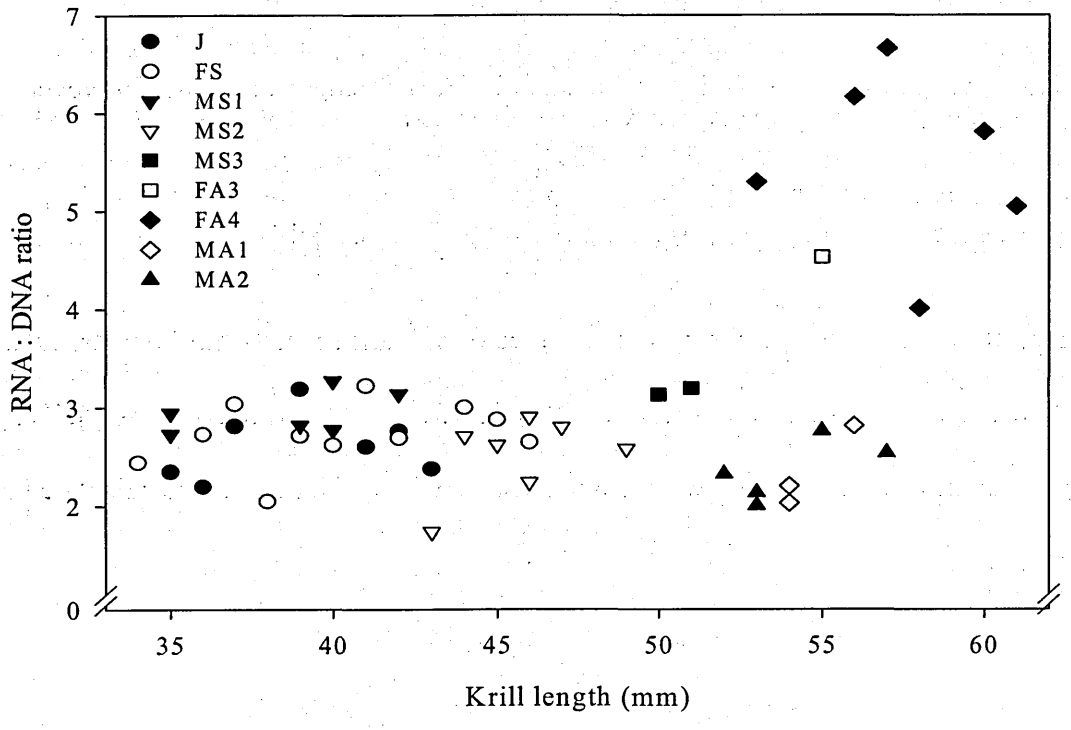

Figure 3.13. Relationship between total length and RNA : DNA ratio in Antarctic krill.

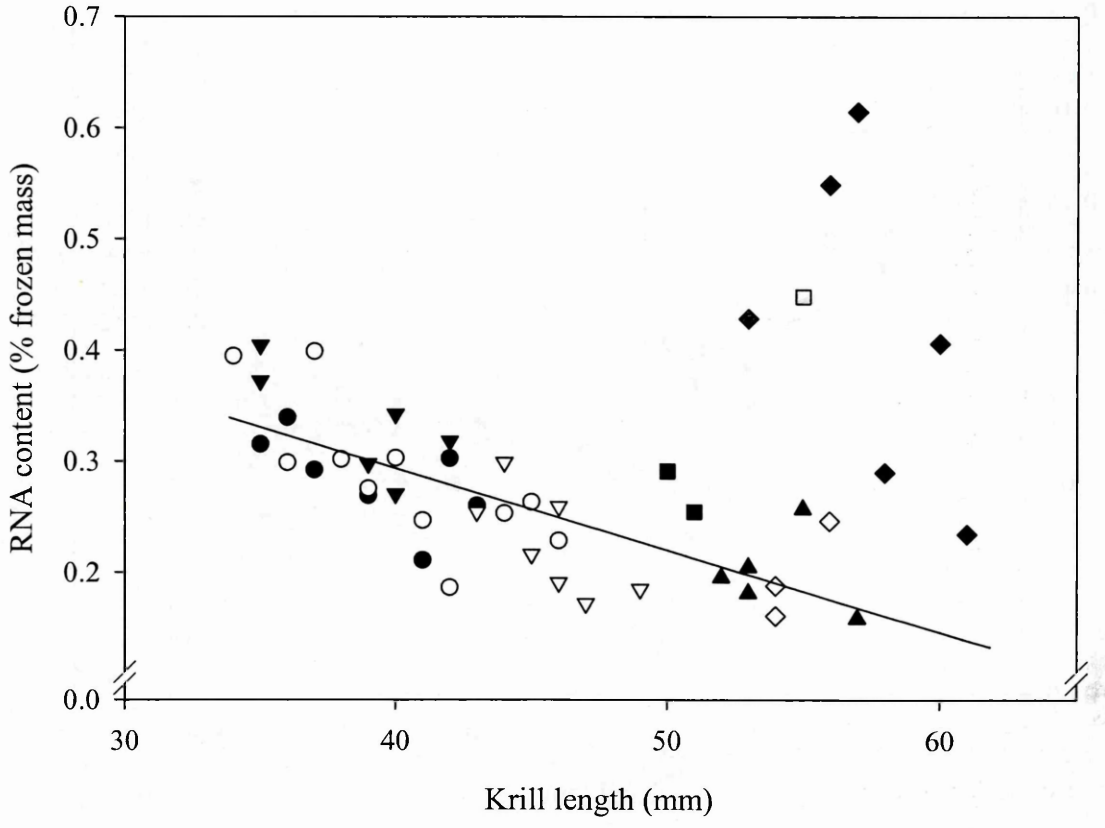

Figure 3.14. Relationship between krill total length (mm) and total RNA content (% frozen mass). Fitted line shown for data excluding FA3 and FA4 animals (gravid females). R^2 = 56.2%, RNA (% frozen mass) = 0.588678 – 0.0073763 length (mm). (Legend as per Figure 3.13).

There was no significant relationship with RNA and length or mass. However, if the gravid female stages (FA3 and FA4) are removed, a significant relationship between length and RNA content emerges (r^2 = 56.2%, p <0.001) (Figure 3.14).

Despite intuition to the contrary, there is a significant relationship between DNA content and length (r^2 = 45.3%, p <0.001) (Figure 3.15) and frozen mass (r^2 = 35.7%, p <0.001). A significant relationship with length remains when DNA content is calculated as a percentage of chitin-free frozen mass (r^2 = 27.8%).

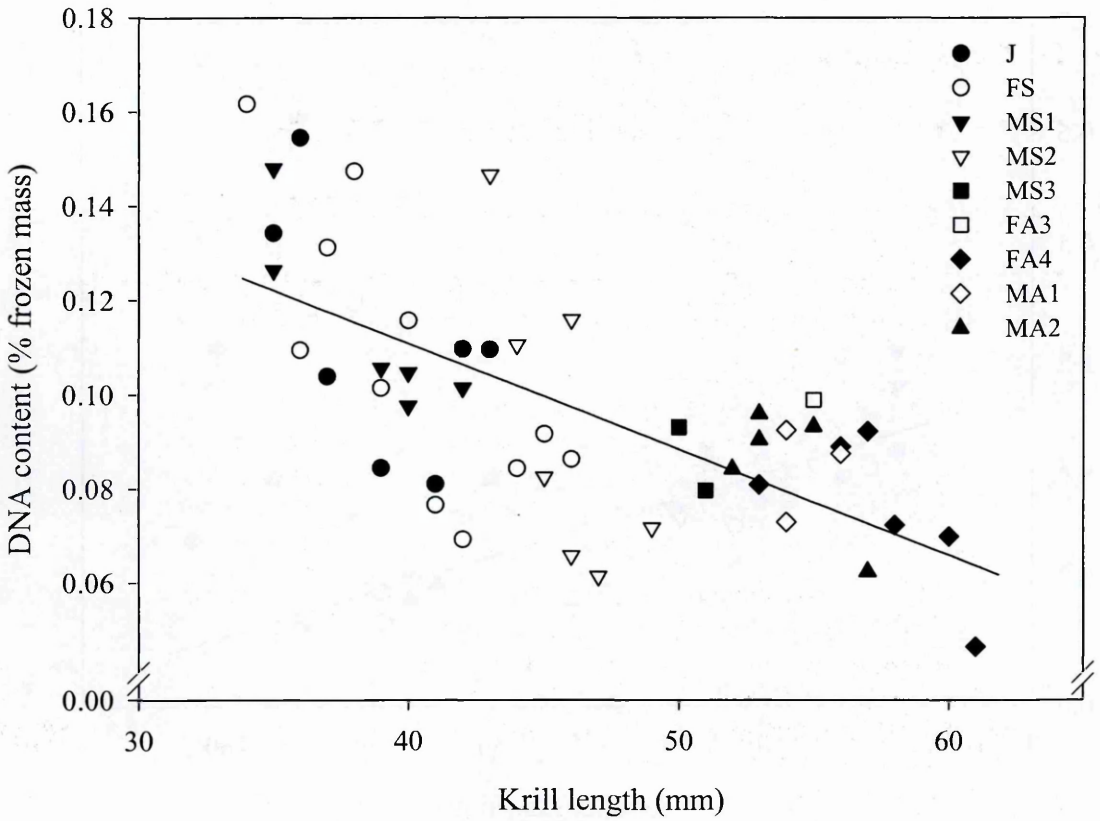

Figure 3.15. Relationship between krill total length (mm) and DNA (% frozen mass), no data excluded. R^2 = 45.3%, DNA (% frozen mass) = 0.200780 – 0.0022457 length (mm).

There was a significant relationship between swarm (ANOVA, $F_{18, 29} = 3.83$, p = 0.001) and sampling location (ANOVA, $F_{15, 32} = 3.90$, $p = 0.001$) and DNA content, but no such relationships with RNA. RNA : DNA ratios also show no correlation with sampling event (swarm) or location. There was a significant difference between both RNA (ANOVA, $F_{1, 45} = 5.91$, p = 0.019) and DNA (ANOVA, $F_{1, 45} = 7.13$, p = 0.010) content of krill sampled on and off the continental shelf, with higher concentrations found off-shelf in both cases. However, there was no difference in the RNA : DNA ratios of on- and off-shelf krill. Significantly higher chlorophyll *a* values were found off the shelf, however only DNA showed a relationship with the chlorophyll *a* values at the sampling location (r^2 = 28.3%, p <0.001), with DNA content increasing with higher chlorophyll concentrations.

Overall the nucleic acid results show:

- DNA content declines with length
- RNA content does the same, except for FA4 and FA5 females
- So, RNA : DNA ratios broadly constant except for FA4/FA5
- In FA3/FA4 there are striking increases in RNA, and hence RNA: DNA ratios.

Chitin

Chitin composition ranged from 0.24% to 4.13%, with a mean of 1.86% and a coefficient of variation (CV) of 51.2%. There was a significant difference between sexual maturity stages (ANOVA, $F_{8, 38} = 2.47$, $p = 0.029$), although not with pooled sex (ANOVA, $F_{4, 42} = 1.93$, $p = 0.123$) (Table 3.20, Figure 3.16). There was no relationship between chitin content and length or mass. There was a significant difference between swarm (ANOVA, $F_{16, 30} = 2.87$, $p = 0.006$), sampling events (ANOVA, $F_{12, 34} = 3.34$, $p = 0.003$) and on- and off-shelf stations (ANOVA, $F_{1, 45} = 9.91$, $p = 0.003$), with krill from on-shelf stations having a higher chitin composition. There was no relationship between station chlorophyll and krill chitin content, although there is a significant difference in chlorophyll between on- and off-shelf stations (ANOVA, $F_{1, 46} = 9.98$, $p = 0.003$).

Table 3.20. Chitin content of Antarctic krill as a percentage of frozen mass. (SE, standard error; *n* = total number of krill analysed). Data in italics is shown for pooled sexual maturity classifications (adult females, sub-adult and adult males).

	Chitin (% frozen mass)				
	Mean	SE	Chitin (% dry mass)	*n*	Length (mm)
All	1.86	0.14	11.91	47	33–61
J	1.24	0.16	8.14	7	33–43
MS1	1.75	0.25	10.89	9	34–45
MS2	2.52	0.56	15.73	5	44–49
MS3	3.62	0.22	21.24	2	47–53
MS	*2.22*	*0.27*	*13.84*	*16*	*34–53*
MA1	2.54	0.32	15.67	3	43–54
MA2	1.69	0.47	12.35	5	51–58
MA	*2.01*	*0.34*	*13.88*	*8*	*43–58*
FS	1.91	0.37	12.27	9	33–46
FA3	1.54	—	9.63	1	51
FA4	1.37	0.10	8.44	6	54–61
FS	*1.40*	*0.09*	*8.61*	*7*	*51–61*

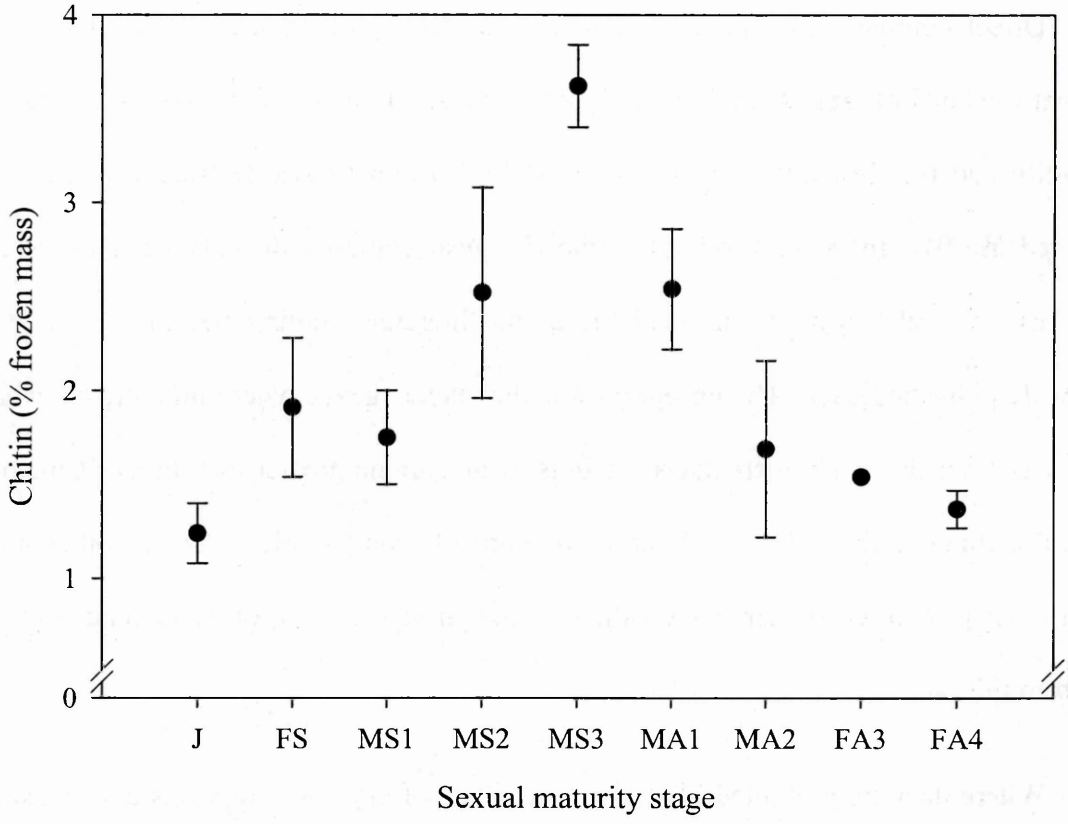

Figure 3.16. Difference in total chitin content (% of frozen mass) between sexual maturity stages in krill from JR70. Values are means ± 1 standard error.

Discussion

Direct comparisons with other studies are made problematic by the plethora of different methods of analysis, range of sampling locations with associated environmental variability and the different classifications used for ascribing sex and maturity stage (if presented at all). In general, however, the chemical composition data from this study compares favourably with those available in the literature, falling within the range of previously published data. The exception was the nucleic acids, where little previous data exist; data from this study were several orders of magnitude greater than that of Cullen et al. (2003), although the RNA : DNA ratios were broadly comparable. The only other study (Ikeda, 1989), reported higher RNA values, although in the same order of magnitude as data from this study.

Where data are presented here as a percentage of dry mass, this was calculated on the basis of a mean water content for each sexual maturity stage as derived by this study. These observed water contents are at the lower end of the range of available data (see Table 3.1). It should be noted that presentation of data as a percentage of dry mass may incorporate slight errors associated with variations in the water content of individual krill.

According to Mauchline (1980a), the wide range of estimates for the water content of krill could be attributed to the fact that the quantification of water content is subject to significant experimental error. It is not possible to test for any influence of experimental technique on water content from the summary data presented, as not enough replication exists. However, it is unlikely that there would be a significant difference between methods involving drying to a constant mass, even when varying temperatures are employed. It is possible that a difference may exist between freeze- and oven-dried samples, though, as it has been shown that oven drying can lead to slightly reduced dry masses (2%) when compared with freeze-dried samples (Postel et al., 2000). Also likely to

contribute to any experimental error would be any extraneous water or salts from the seawater remaining on the animal when the fresh/frozen mass was determined. Additionally, there is the possibility that frozen mass is slightly higher than fresh mass, which may explain the lower mean from this study.

Another factor which can significantly influence water content is the stage at which an individual krill has reached in its moult cycle at the point of sampling. Clarke and Lakahani (1979) observed seasonal variation in the water content of the benthic prawn, *Chorismus antarcticus* which they attributed to variation in moulting frequency and Nicol et al. (1992a) showed a higher water content in krill at the point of moulting, than at any other point in the moult cycle. Unfortunately, the krill used in the biochemical analysis were not individually moult staged (for reasons explained in Chapter 2), so we cannot definitively test the idea that moult stage is a factor affecting water content. However, we can look at the classification ascribed to the swarm for those individual krill that were moult staged. For both cruises considered in this study, moult stage measurements were carried out by Geraint Tarling using the method of Buchholz (1991) on the majority of krill swarms caught. This analysis was undertaken to determine if there was synchronous moulting occurring in the swarms. The principle of this method considers that the moult cycle is divided into distinct stages of unequal durations. A global assessment is made of all the krill ascribed a moult stage by this method during the sampling season, assuming that the division of the stages represents the time an average krill spends in each phase of the moult cycle (or, the proportion of a given population that will be in that phase of the moult cycle at any given point in time). For each swarm, the division of stages is tested against the expected global division using a chi- squared test; if there is a significant difference from the expected result, a moult stage is ascribed to that swarm which contributes most of the variation from the expected divisions, i.e. one moult stage can be said to 'dominate' the swarm (although, numerically this may not be the case). These

ascribed moult stages were tested to see if they contributed to a difference in water content in the krill analysed. A significant difference was observed in water content with dominant swarm moult stage (ANOVA, $F_{7, 218} = 4.99$, $p > 0.001$), with the highest water content found in krill from swarms where the post-moult classification dominates (Figure 3.17), supporting the findings of Nicol et al. (1992a). This analysis does, of course, assume that the krill used for water content determination were representative of the whole swarm.

The relative order of magnitude of the proximate components agreed with previous studies, with protein being the largest component (with the exception of water), followed by lipid, mineral ash, chitin, carbohydrate, RNA and DNA respectively. With the exception of chitin, this sequence is typical for any marine invertebrate; including chitin makes it typical for a crustacean.

Proximate components differed in their individual variability, with the highest CV seen in chitin, followed by lipid, RNA, carbohydrate, DNA ash, protein and water respectively. Raymont et al. (1971) and Torres et al. (1994) describe lipid as the most variable fraction, with Torres and co-workers describing high levels of variability in individuals from the same net haul for both lipid and protein, in addition to that seen with space and time. It is not altogether surprising that such high levels of variability were found in the first three components, as physiologically these are the fractions that we would expect to undergo the most variation throughout the lifecycle of krill.

Variability in chitin content between individual krill is almost certainly linked to the moult cycle. Krill undergo a cycle of moulting that lasts approximately 20 days (Quetin et al., 1994) during which the exoskeleton is shed and a new one built and strengthened post moult before being partially reabsorbed prior to loss as an exuvae at the next moult (Buchholz, 1991; Nicol et al., 1992a). The intermoult period (IMP) is temperature dependant, with krill moulting more frequently at higher temperatures (Quetin

et al., 1994), and has recently been shown to vary depending on sex and maturity stage (Tarling et al., 2006). Chitin composition was compared with mean moult cycle classifications for each swarm, as described above, showing a significant difference in chitin content with moult stage (ANOVA $F_{6, 40}$=2.64, p=0.030), with the highest levels of chitin found in mid pre-moult krill, and the lowest levels in the soft late pre-moult stage (Figure 3.17). This must be treated with some caution, however, as individual krill were not moult staged (as discussed above). However, a detailed study by Nicol et al. (1992a) has shown significant changes in chitin composition with time since moult. Similarly, ash content has been shown to have a dramatic increase in concentration immediately after moulting (Nicol et al., 1992a) as the exoskeleton is strengthened by the process of mineralisation of the chitin matrix.

Lipid levels change as animals lay down lipid as a storage reserve, the degree as to which krill use lipid as a storage mechanism have been much debated, however recent work by Hagen et al. (2001) shows clearly that a late summer increase in lipid content is an important factor in overwintering energetics. Also, lipids are crucial components in reproductive physiology (Cuzin-Roudy & Amsler, 1991; Mayzaud et al., 1998b).

It is likely, however, that the majority of variation in the mean proximate composition data can be explained by the mix of sexual maturity stages included in the data.

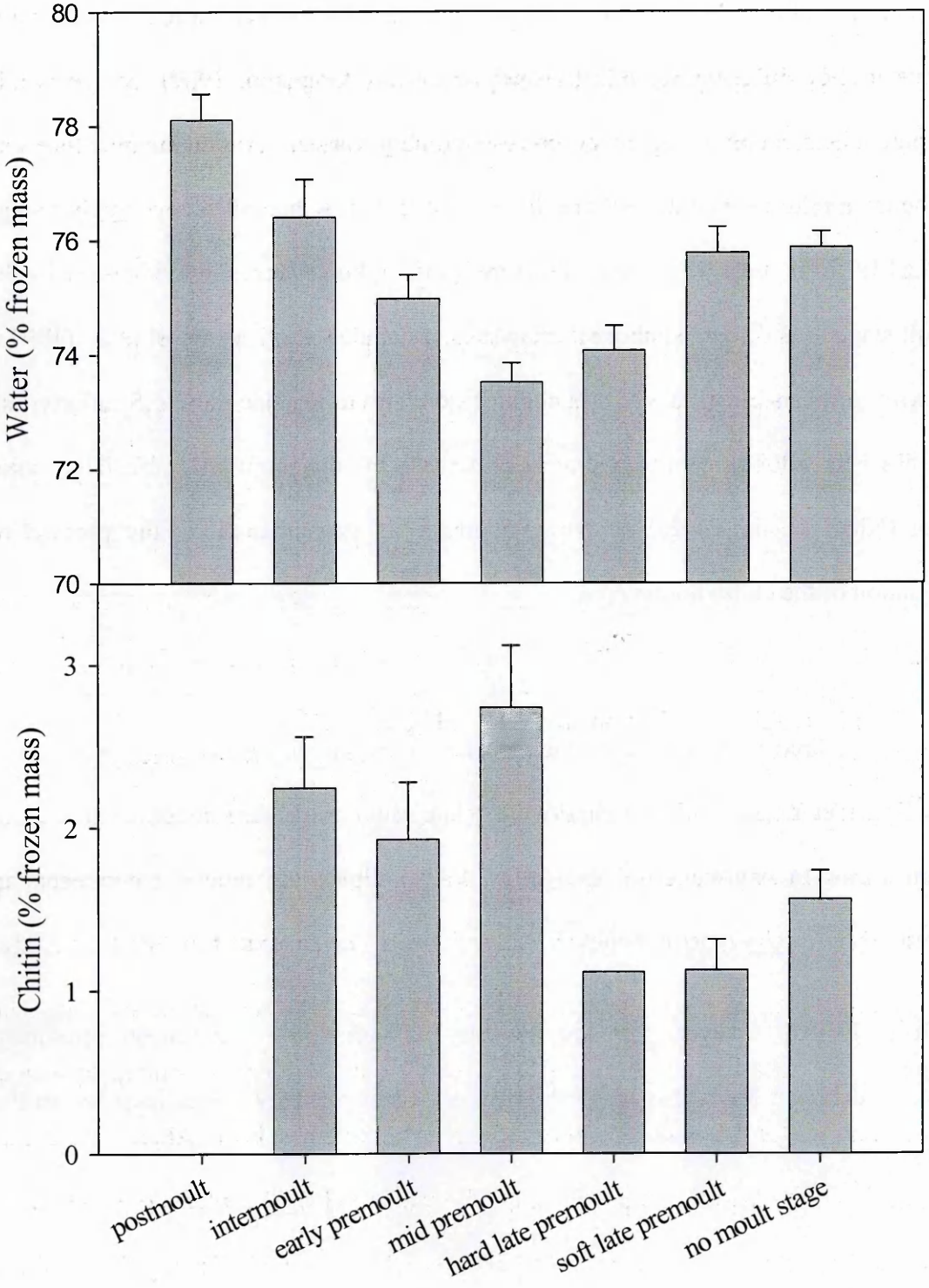

Figure 3.17. Variation in water and chitin composition with moult stage. 'No moult stage' data includes individuals from swarms where no dominant moult stage was detected, or individuals not checked for moult stage. There were no postmoult krill in those selected for chitin analysis. Values are means ± 1 standard error.

Variation with sexual maturity stage

Mature, adult krill show striking morphological differences between the sexes and they have widely differing reproductive physiologies (Bargmann, 1937), so we would expect their chemical make up to be correspondingly varied. Although immature and juvenile animals show few outwardly visible differences, as they develop, we might expect that the physiological changes during maturation to produce detectable changes in the proximate composition between maturity stages. Indeed, all components with the exception of DNA showed a significant difference in content between sexual maturity stages. Generally differences were less significant when the pooled classifications were considered. This could be the result of a reduced replication or that pooling masks clear differences between sequential stages, for example the large increase in protein between MA1 and MA2 (pooled together as adult males in the reduced classification). Only differences with the full classification are discussed here.

It is difficult to provide a definitive explanation for the differences in water content between sexual maturity stages, although data from both cruises show the same general pattern of a decrease in water content with maturity. It is possible that mature krill do not moult as frequently as the growing juvenile and sub-adult stages, and therefore do not take on seawater as frequently, resulting in a lower mean water content, supported by recent work of Tarling et al. (2006) which showed higher IMPs in larger krill. Alternatively, this could be attributed to the inverse relationship with water and lipid contents (a comparison of mean data from this study is consistent with this relationship), indicating that krill are laying down more lipid stores as they mature. Potentially, reproductively active males could be using up lipid stores producing spermatophores, although it is thought the investment in sperm is small. However, the energetic costs associated with the transfer of spermatophores to the females could be high, especially as a male may mate several times in a season. This is consistent with the striking low value for lipid in krill from the

spermatophore producing stage (MA2) and the high water content. The high value for FA5, post-spawn female krill represents the physiological change at the point of spawning between swollen ovaries enlarging the thorax and the large fluid filled space that remains until the next moult, once the eggs have been released (Cuzin-Roudy & Amsler, 1991). A high mineral ash content in FA5 is also indicative of the thorax cavity becoming filled with fluid. A significant positive relationship has been observed between water content (% frozen mass) and mineral ash content (% dry mass) in this study (Figure 3.18), which would suggest a correlation between an increase in ash being linked with an intake of seawater post-moult or post-spawn with its high concentration of minerals. From these chemical data we can assume seawater enters the void in the thorax left after spawning in mature females.

Mineral ash content was shown to mirror the trends in water content between the sexual maturity stages. In addition to the minerals found in seawater, internal fluids have a high ionic strength and these salts are also left behind as ash after combustion. However, as the relationship between water content and mineral ash proved to be positive, suggesting the majority of the water in krill comes from seawater intake rather than water from cellular metabolism, this was to be expected.

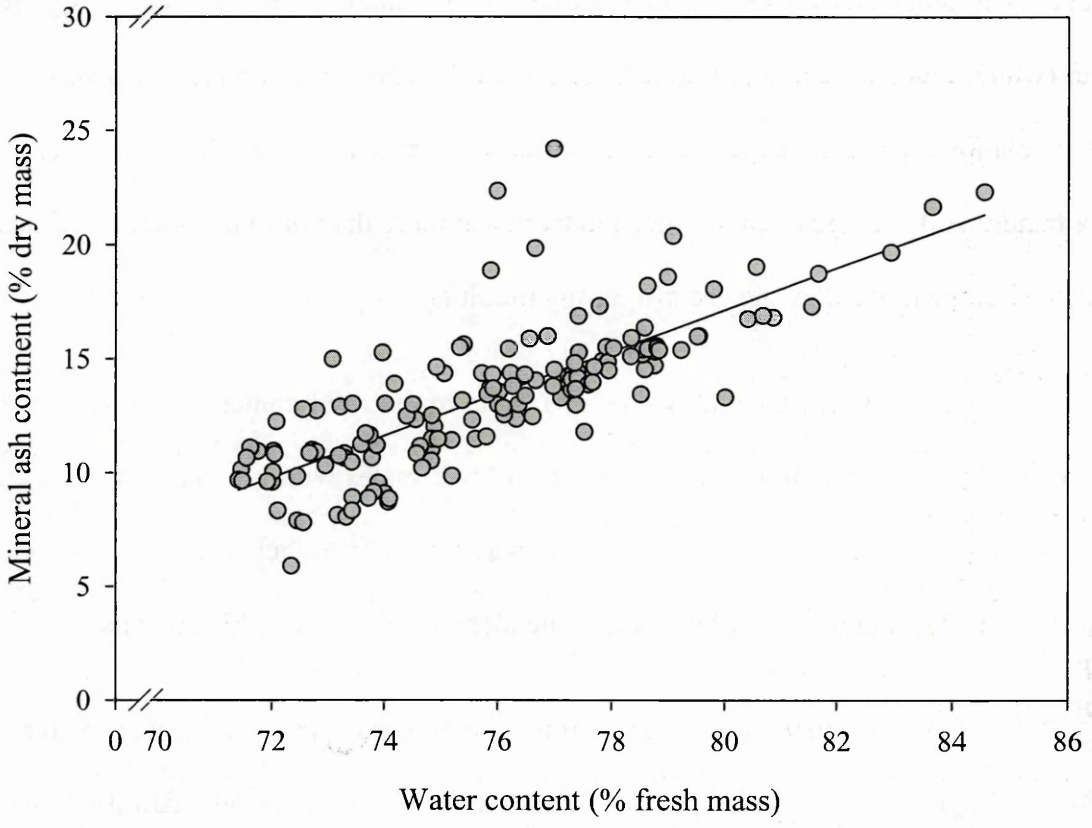

Figure 3.18. Relationship between water and mineral ash content in Antarctic krill. R^2 = 61.6%, mineral ash (% dry mass) = -56.4865 + 0.920371 water content (fresh mass).

Clarke (1984) presented a review of studies on the lipid composition of Antarctic krill, and concluded that mature females contain more lipid than mature males (except possibly in the late summer). However, the data from this study did not show mature and gravid females to have a higher percentage lipid than the sub-adult and pre-spermatophore adult male krill, although gravid female krill contained by far the highest amount of lipid per individual (due to their swollen thorax packed with eggs). Unfortunately, no post-spawn krill were available for my analysis, so the loss of lipid after spawning was not assessed here, although (Clarke, 1984) showed a loss of 54% of total lipid at spawning (a reduction from 6.01 to 2.79% lipid). Adult males that have produced spermatophores, however, had a much lower lipid composition than any of the other stages. After spermatophore transfer the males are characterised by extremely low total lipid and a very high mortality (Pond et al., 1995; Virtue et al., 1996), although the animals are not

starving. This indicates that the cost of reproduction is high in males as well, with an energetic cost associated with the production of spermatophores and spermatophore transfer (which involves catching a female first!). Although speramatophore 'packets' are small in relation to the dramatic increase in size of a gravid female that has produced approximately 100,000 eggs, females need to be mated more than once as speramatophores are attached to the thyleculm and are lost during moulting.

This study showed remarkably similar results in the lipid content of FA4 females compared with Clarke's mature females, and with MA2 males with his mature males, but the lipid content of juveniles found by this study was markedly higher. On the other hand, Hagen et al. (1996) found no significant sex-dependent differences within seasons.

Zhu and Wang (1989) showed that protein content increased with sexual maturity of krill (although krill analysed were not individually ascribed sexual maturity stages, rather a modal swarm value data used to classify maturity). This is broadly supported by data from this study, which showed a marked increase in protein from the final sub-adult stage to fully mature animals in both males and females. This could be indicative of the animal gaining muscle mass as is grows in length. However, for the progression from juvenile to sub-adult (an increase in length) there is an apparent decrease in protein content. Potentially, this could result from a transfer of resources, from muscle growth need in juveniles, to the development of reproductive structures and secondary sexual characteristics in the sub-adult animals. Once these are developed, energy is again available for muscle growth.

Carbohydrate exhibits a similar increase in content with increasing maturity, with the anomaly of a marked decrease in carbohydrate concentration between male sub-adult stages 1 and 2 and then an increase to maturity. There would appear to be no obvious explanation for this change in carbohydrate between these two stages, when the change in

external secondary sexual characteristics would not appear to require significant investment. Carbohydrate is not a very efficient source of energy in the short term, as it needs to be broken down to release its energetic potential and it is unlikely in this instance that it is being used as a reserve in the absence of sufficient lipid, as MS2 krill has a higher percentage lipid content than MS1. Mature females had a higher carbohydrate content than males, in agreement with Zhu and Wang (1989).

RNA contents were higher in the early juvenile and sub-adult stages and markedly higher in mature female stages, than the later male adult and sub-adult stages. This is likely related to the potentially higher growth shown in rapidly growing young krill and reproducing females, producing oocytes and increasing body size to accommodate this enlarged ovary. DNA shows no significant difference with sexual maturity stage, despite a general trend for decreasing DNA content with increased maturity. This showed as a slight significant difference with pooled stage. RNA:DNA ratios showed a significant difference with sex, purely because FA3 and FA4 krill had a significantly higher RNA values than all other stages (Figure 3.13), whilst the ratio across all other sexual maturity stages is broadly constant. Cullen et al. (2003) also demonstrated higher RNA : DNA ratios in female krill. An increase in RNA in the mature female stages would be essential to achieve the rapid growth involved in the production of oocytes and dramatic swelling of the thoracic cavity to accommodate the enlarged ovary.

Morphometric variation

Length and body mass also have the potential to affect the chemical composition of the krill, although sexual maturity stage and size are linked; more mature animals are typically larger and heavier, and inherent differences in composition attributed to sexual maturity stage may cloud any relationship with size.

Not all proximate fractions showed a correlation with length and/or mass for all data pooled, but in some cases (where sufficient data allowed analysis) there were significant relationships between animals of the same sexual maturity stages. For example, water content was not correlated with length for all krill, but when each sexual maturity stage is considered separately, there is a negative relationship with length in juveniles. This could result from lipid accumulation by juvenile krill, maximising available food resources to provide energy for growth. Although lipid data showed no relationships when all data were considered, when the sexual maturity stages were analysed separately, juvenile and young sub-adult males (MS1) showed an increase in lipid with length. Ferguson and Raymont (1974) have shown an increase in lipid content with size, but do not sort by sex, although their data contained mostly juveniles. Mature males (MA2) showed a decrease in lipid with length, which could be explained by the larger krill been more reproductively active, for longer, than smaller krill. It is possible, but speculative, that the larger animals are more successful at mating female krill (faster swimming speed, more experienced) and so could have transferred more spermatophore packets to females, which, as discussed previously, results in a depletion of lipid. However, work by Mayzaud et al. (1998b) showed that neither body mass nor size are the main factors controlling lipid accumulation in krill, consistent with Clarke (1980) who failed to demonstrate a relationship with size.

Protein content was shown to increase with length and frozen mass when all krill are pooled, indicating an increase in muscle with size, as discussed previously. This contradicts findings by Ferguson and Raymont (1974) which suggest that protein content may be relatively higher in smaller krill, especially earlier in the summer. A negative relationship with mineral ash and length was shown, most significantly in krill sampled during JR82 which could be attributed to a decreases in moulting frequency (and intake of seawater at moult) as the animal grows larger (although not supported by the work of

Tarling et al. (2006) conducted during JR70 and JR82, who showed IMP increased with body size), or the artefact of the relative amount of water taken on post-moult compared to the size of the krill.

Perhaps the most interesting relationship with length was the significant negative correlation with length and DNA composition in krill. It is usually assumed that the DNA content of cells is constant; therefore DNA content can be considered a proxy for biomass. Therefore, a relationship between DNA and length would not be expected. However, Figure 3.15 clearly demonstrates that, for krill, such a relationship does exist. The most likely explanation for this relationship is that, rather than increasing cell number at growth, it is instead the cell volume that increases. This has been shown to occur in fish muscle (Johnston et al., 1999), with salmon muscle cells enlarging muscle fibres, changing the size of the muscle cell without increasing the amount of DNA present. This is also to some degree supported by a study by McGaffin et al. (2002) who showed that experimentally shrunken krill (i.e. food limited) contained a higher density of muscle nuclei compared with freshly caught adults. This could be interpreted as in times of stress, krill can reduce the volume of muscle cells, so we assume that they increase during good food conditions and periods of growth. With the exception of the adult females (as previously discussed), RNA also displays a negative relationship with length. Because DNA and RNA are both decreasing with length, there is no relationship with RNA: DNA ratio and length, and it is only the high RNA values in mature female krill that cause them to exhibit the greatly elevated ratios.

Based on these findings it is not possible to describe the general proximate chemical composition of krill as a simple function of length or mass alone. What relationships do exist are rendered complex by effects of sexual maturity.

Effects of environmental variability

Animals used in the proximate composition analysis were sampled from waters with varying chlorophyll concentrations and so the data were examined to see if these different conditions lead to patterns in the chemical make up of the krill. Chlorophyll values used in the analysis were surface concentrations obtained by CTD cast (6.5–7 m depth samples), where a krill sampling event is assigned the value of the nearest CTD cast in space and time (Appendix C).

There was a significant difference in the DNA content of different swarms and sampling events, with a higher concentration of DNA in krill sampled in areas of high chlorophyll concentrations. Cullen et al. (2003) showed a strong relationship between RNA : DNA ratio and food availability in agreement with this study. High nucleic acid ratios in areas of high chlorophyll concentrations is an indication that the krill are increasing protein synthesis in response to the available food (see previous page).

Chitin concentrations showed significant differences between swarms, and also on-/off-shelf location with samples from the shelf displaying higher proportions of chitin. Although there was no correlation between chlorophyll concentrations and sampling event, off-shelf locations displayed higher chlorophyll. The most likely explanation is that animals grow faster in areas of higher food availability, thus having a higher moulting frequency and perhaps, as a consequence, lower levels of chitin. The difference between swarm and event is likely to be attributable to the moult cycle, whether it is influenced by food availability or not; interestingly there was a stronger relationship with chitin concentration and swarm than sampling event, which suggests the moult cycle is more important in determining chitin concentration than aspects relating to food availability. It is possible that the swarms are moulting synchronously, with krill from the same swarm all at the same point in the moult cycle.

Carbohydrate showed a significant difference between events and on- and off-shelf samples (with a higher concentration of carbohydrate in krill sampled off the continental shelf), yet these difference did not correlate with observed chlorophyll values. It is possible that there is some other environmental factor influencing the carbohydrate in animals from different locations, although it is more likely that these differences are an artefact of the differing composition of sexual maturity stages analysed from each location. Similarly, lipid showed differences between swarms and also sampling events which were not related to chlorophyll concentrations.

However, especially for the animals sampled during JR70 from around South Georgia, local conditions can vary quite quickly and as the animals more mobile, both in the water column, and by transport in the larger currents, they can be passing quickly through areas of differing environmental conditions, and their chemical composition may reflect conditions encountered several days or weeks previously, if at all.

Spatial and temporal variability

For the proximate composition analysis, only water content and mineral ash were determined in krill from both sampling cruises (different years and locations).

Differences in water content between cruises and between published data could be attributed to sampling environmental conditions, for example ice cover. Torres et al. (1994) showed that there was a statistically higher water content in krill sampled from consolidated pack ice (mean 79.5, n = 16) than from nearby open water (mean 75.2, n = 16) in winter, but not in autumn. Ice was encountered in JR82 (with higher mean water content) but not during JR70, although a direct correlation with the presence of ice at or near the sampling station and water content was not found. However, the region of the Southern Ocean sampled in JR70 around South Georgia is not subject to seasonal sea ice, unlike much of the sampling area from JR82 (see Figure 1.1).

It is more likely, however that the differences in mean water content between years is related to the observed relationship with water content and latitude, as the geographic area sampled in the two cruises was very different. Although the water content of krill sampled during JR82 was higher than that sampled on JR70, a comparison of the means of animals sampled from the same geographical area showed no significant difference in the means. This suggests that location, rather than 'year effects' was contributing to the variation in this case. Significantly, water content from this cruise correlated with location, with krill from higher latitudes and *more westerly* longitudes having higher water contents. This increase with water content from locations further towards the Antarctic Peninsula is intriguing, and is most likely attributable to the differing food and temperature conditions encountered by the krill during the previous months, with colder temperatures and lower concentrations of chlorophyll typically found further south. Although there was no observed correlation with water content and chlorophyll concentrations, a significant relationship was shown with chlorophyll content and longitude of sampling stations, with concentrations increasing from west to east. High water contents could reflect potentially lower lipid contents in these animals caught in areas with less food. Alternatively, it could be that these higher water contents in animals from further south and east, could relate to the fact that animals from these locations were typically smaller (significant relationship with latitude and longitude and length). Although there was no relationship with length overall, juvenile animals did show a trend to lower water contents with increasing length. When results from the literature Table 3.1 are considered in this context, no pattern emerges as there is not sufficient resolution in the data to allow a detailed analysis.

However, it is apparent that season affects water content in krill, with Cullen et al. (2003) describing higher water content in late spring than in summer and autumn. Similarly Torres et al. (1994) showed a higher water content in winter than early autumn, and Clarke (pers. com) found more water in male krill in summer than winter. This

indicates a potential seasonal cycle in krill water content, with a peak in spring, falling to a low in autumn and rising again over winter. There are two possible explanations for this seasonal pattern: variations in lipid content and seasonality in the moult cycle, although they are not necessarily mutually exclusive. Falk-Petersen et al. (2000) showed a seasonal trend in total lipid with krill having a peak in lipid in the autumn after a summer of feeding and laying down lipid reserves. This stored lipid is then depleted over the winter to reach a spring low before the start of the next season. A correlation between water content and total lipid has been observed in fish (Craig et al., 1978; Henderson et al., 1984) and in Northern krill (*Meganyctiphanes norvegica*) (Falk-Petersen, 1985); which can be explained by the oxidation of a fatty acid molecule generating an equal volume of water (Falk-Petersen et al., 2000). There has been some suggestion of changing water content associated with the moult cycle; Nicol et al. (1992a) observed a 3.9% change in water content during the moult cycle, with the highest amount of water found immediately post moult, and a decline through the following moult stages. All crustaceans, including krill, take on water immediately after ecdysis to enable growth (Forest & Von Vaupel Klein, 2004). However, the change in water content attributed to the moult cycle, inherent in all studies of water content in krill, cannot be quantified without the moult staging of individuals.

Differences in mineral ash content between cruises are likely to be driven by the changes in water content, as it was shown that water content and mineral ash were correlated (Figure 3.18).

Lipid content has also been shown to be highly seasonal; Raymont et al. (1971) showed higher lipid in January than December, concurring with the findings of Kryuchkova and Makarov (1969a) that lipid content tends to increase throughout the summer season and Suzuki and Shibata (1990) who present data which indicates an increase in lipid for each month from December to February. Hagen et al. (1996) showed

that lipid accumulation was highest in the autumn, reaching a minimum in spring after depletion over the winter. Summer levels were more variable, but intermediate, indicating an accumulation over the productive season to reach maximum in autumn. They found no significant sex-dependent differences within seasons, but attributed the higher variability during the summer to reproductive processes, such as egg production, which only occurs in summer (Cuzin-Roudy, 1993).

Torres et al. (1994) showed a significant decrease in lipid from animals sampled from within pack ice, than those from nearby open water in autumn and winter. Within a season, Clarke (1980) and Pond et al. (1995) observed spatial differences in lipids, but they were not able to correlate this variation with phytoplankton availability.

ELEMENTAL CHEMISTRY

Introduction

Elemental composition has been previously investigated in *Euphausia superba*, by several authors, with the aim of answering targeted questions, for example aspects of the animals ecology such as moulting (Nicol et al., 1992a) and seasonality (Torres et al., 1994) or metabolic physiology (Ikeda, 1984, 1985a; Ikeda & Bruce, 1986). In addition elemental composition (particularly carbon) is frequently used as a biomass measure (Huntley et al., 1994; Hofmann & Lascara, 2000).

Methods

Carbon, hydrogen and nitrogen

The elemental analysis of carbon, nitrogen and hydrogen is a frequently used technique in studies on zooplankton for a range of applications (Clarke et al., 1992; Gorokhova & Hansson, 2000; Jaenike & Markow, 2003) since the development of automated techniques that allow all three elements to be determined on small amounts of tissue. Many authors have investigated the elemental composition of krill (see Table 3.22), with the only significant difference in methodology being the make of analyser used.

After drying to a constant mass (60 °C, 48 hours), krill were individually homogenised with a pestle and mortar. Triplicate sub samples of approximately 1 mg were taken from each animal, placed in a pre-weighed tin capsule and the mass determined to ± 1 μg on a microbalance (Mettler MT5). Sealed capsules were analysed for carbon, nitrogen and hydrogen content in a CE Instruments EA 1108 CHN elemental analyser. Acetanilide was used as the standard and the instrument was calibrated with a five point curve at the start of each daily run of analysis, with duplicate standard samples analysed every ten krill replicates to check that no drift from the calibration had occurred.

Because krill, at up to over 1 g dry mass, were too large to be analysed whole in any CHN elemental analyser, sub sampling must be undertaken. It was imperative that samples were adequately homogenised before sub sampling, but with the optimum mass for analysis of krill tissue determined as ~ 1 mg for the particular machine used, the question remained how many replicates were necessary to compensate for the variability inherent in small subsamples of a large, complex organism. In order to determine the exact number of replicates necessary to remove this variability, 25 consecutive replicates of a single, homogenised krill were analysed (duplicate standards run every ten samples). The variance proportion of the variance contributed by each successive replicate was investigated (variance described as between sample variance and within sample variance divided by the mean), along with the standard error and standard error as a proportion of the mean for each of the successive 25 replicates, for each element. Results are presented in Table 3.21.

The variance dropped sharply with increasing replication, with the decrease slowing markedly after three replicates. As with any analytical technique there is a trade-off between precision and analytical through-put. Since this project required a large number of individuals to be analysed to detect biological and environmental variation, it was decided to use 3 replicate sub-samples for each individual krill. This reduced a significant proportion of the variance and was feasible in terms of time and cost considerations (each sample and standard took 10 minutes to analyse and cost approximately £1).

Table 3.21. Contribution to the variance of the mean for each element for any given number of replicate subsamples in the elemental analysis of homogenised krill. (SE = standard error).

	Number of replicates	Variance	Standard error	SE as % of mean
Carbon				
Mean 48.155	1	10.109	10.109	20.993
	2	10.011	5.055	10.496
	3	9.978	3.370	6.998
	4	9.961	2.527	5.248
	5	9.951	2.022	4.199
	10	9.932	1.011	2.099
	25	9.920	0.404	0.840
Nitrogen				
Mean 9.522	1	0.885	0.885	9.294
	2	0.877	0.443	4.647
	3	0.874	0.295	3.098
	4	0.872	0.234	2.457
	5	0.871	0.177	1.859
	10	0.870	0.089	0.929
	25	0.869	0.035	0.372
Hydrogen				
Mean 7.408	1	0.178	0.178	2.403
	2	0.175	0.089	1.201
	3	0.173	0.059	0.801
	4	0.173	0.045	0.601
	5	0.172	0.036	0.481
	10	0.172	0.018	0.240
	25	0.171	0.007	0.096

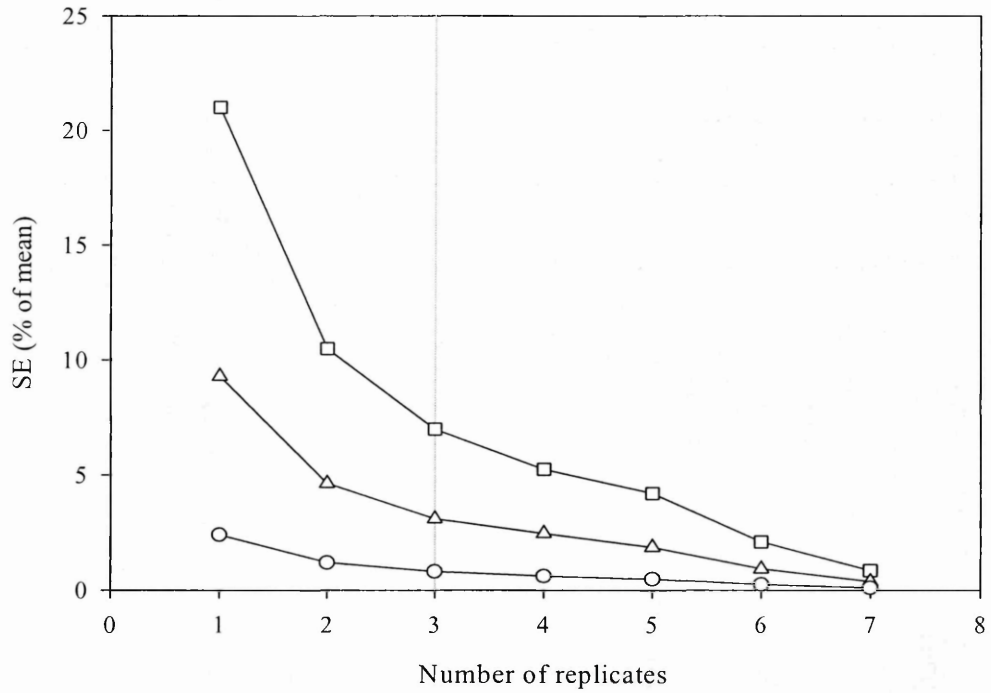

Figure 3.19. Change in standard error (as a percentage of the mean) with number of replicate analysis. Carbon shown as squares, nitrogen as triangles and hydrogen as circles. Solid line shows number of replicates selected.

Table 3.22. Summary of elemental composition data (carbon, hydrogen and nitrogen) for *Euphausia superba* available in the literature (% dry mass). Measurements on whole animals unless otherwise stated. All krill masses presented as dry mass. (SE, standard error; *n* = total number of krill analysed; * ± 95% confidence limits).

Study	Analysis method	Antarctic location	Time of year	Krill analysed	Carbon ± SE	Hydrogen ± SE	Nitrogen ± SE	C : N	*n*
Yanase (1974)	—	—	—	—			10.76		—
Clarke (pers. comm.)	Carbo Erba Elemental analyser, acetanilide standard	Palmer Station	Dec	Male	40.97±0.54	6.47±0.08	11.03±0.24	3.71	5
			Winter	Male	46.37±0.27	7.24±0.12	10.25±0.72	4.52	3
				Female	45.07±0.64	6.84±0.05	9.94±0.32	4.53	6
Torres et al. (1994)	Control Equipment Corporation Model 240XA (AFDM)	NW Weddell Sea	March	30–53 mm	49.2±0.77		9.9±0.25	4.97	23
		S Scotia Sea	Winter	20–53 mm	48.3±0.64		11.4±0.23	4.24	32
Ikeda (1984)	Perkin Elmer 240, freeze dried material	Off Wilkes Land	Jan	Juvenile (10–50 mg)	41.1±4.7*		11.0±1.2*	3.74	3
				Juvenile (50–100 mg)	44.7±2.1*		10.2±0.6*	4.38	4
				Adult (100–200 mg)	46.6±1.7*		9.9±0.9*	4.71	5
				Adult (200–300 mg)	47.5±2.2*		10.2±1.1*	4.67	5
				Gravid females (>300 mg)	47.5±2.5*		10.3±0.7*	4.61	3
Ikeda & Bruce (1986)	—	—		47–243 mg	43.9		10.8	4.06	
Ikeda and Kirkwood (1989)	Perkin Elmer 240 acetanilide standard	Off Enderby Land	Nov	Juvenile (< 50 mg)	40.72±0.17		11.99±0.09	3.40	21
				Juvenile (50–100 mg)	40.15±0.09		11.84±0.04	3.43	29
				Adult (100–200 mg)	40.67±0.18		11.85±0.16	3.43	14

Study	Analysis method	Antarctic location	Time of year	Krill analysed	Carbon ± SE	Hydrogen ± SE	Nitrogen ± SE	C : N	*n*
Ikeda and Dixon (1982)	Perkin Elmer 240 acetanilide standard	—	Jan	Unknown	43.1±0.39		10.6±0.10	4.07	38
Ikeda and Mitchell (1982)	Perkin Elmer 240 acetanilide standard	Off Wilkes Land	Jan	27.03±14.16 mg	41.1		11.0	3.74	20
				76.77±16.54 mg	44.7		10.2	4.38	17
				142.86±27.07 mg	46.6		9.9	4.71	26
				237.68±21.51 mg	47.4		10.2	4.65	7
				353.78±26.36 mg	47.5		10.3	4.61	7
Huntley et al. (1994)	Perkin Elmer 240 acetanilide standard	Peninsula	Dec–Jan & July–Aug	All krill				4.76±0.053	92
				2.7–30 mg	34.8		7.64	4.59	—
				30–188 mg	40.3		8.23	4.91	—
			Summer	All krill				4.64±0.069	13
			Winter					4.79±0.057	79
Ishii et al. (1987)	Yanaco CHN Corder, MT-3	—	Summer	11.2–19.6 mg	36.97±0.57		10.25±0.22	3.63±0.07	21
				28.4–49.7 mg	42.52±1.24		10.14±0.31	4.21±0.11	8
				81.6–112.1 mg	41.75±1.49		9.57±0.45	4.40±0.16	7
				105.6–269.8 mg	43.07±1.78		9.78±0.40	4.42±0.15	9
				173.4–360.0 mg	44.30±0.93		9.52±0.25	4.70±0.15	18
Atkinson et al. (2002)	Carbo Erba Elemental analyser, acetanilide standard	SW Lazarev Sea	April	Juvenile	51±0.22 (41–54)		8.6±0.10 (7.0–*??*)	5.93	34
				Adult	54±0.33 (46–57)		7.9±0.11 (7.1–8.8)	6.84	21

Phosphorus

Triplicate subsamples of ~ 3 mg were also taken from each homogenized krill used in the CHN analysis (where sufficient material remained) and analysed for phosphorus content with the method of Fiske and SubbaRow (1925). Samples were digested in 0.8 ml 70% perchloric acid ($HClO_4$) at 180 °C using long Pyrex© test tubes to allow refluxing of the acid and no loss of volume (the digest acts as a constant boiling mixture). Digestion continued for 30 minutes after refluxing started, converting all phosphorus into orthophosphate. A calibration curve was prepared, using potassium dihydrogen orthophosphate (KH_2PO_4) as a standard. After cooling, 4.15 ml of water, 0.5 ml 5% ammonium molybdate ($(NH_4)_2MoO_4$) and 0.4 ml reducing agent (60 g sodium metabisulphite ($NaHSO_3$), 12 g sodium sulphite (Na_2SO_3) and 1 g 1,2,4-aminonaphthol sulphonic acid ($C_{10}H_9NO_4S$) in 500 ml water) was added to the samples and standards and vortex mixed. A further 4.15 ml of water was then added and the tubes mixed again and left to stand for 20 minutes. Once the colour had developed, samples and standards were read in a spectrophotometer at 660 nm and quantified by comparison with the known standards (see Figure 3.20 for an example of a typical calibration curve).

In order to determine the optimum mass of dry material to use for each sub-sample in the assay, a range of masses of homogenate from one individual were analysed from 1–6 mg. It was found that using 3 mg of dried material gave absorbance readings that fell in the middle of the calibration curve.

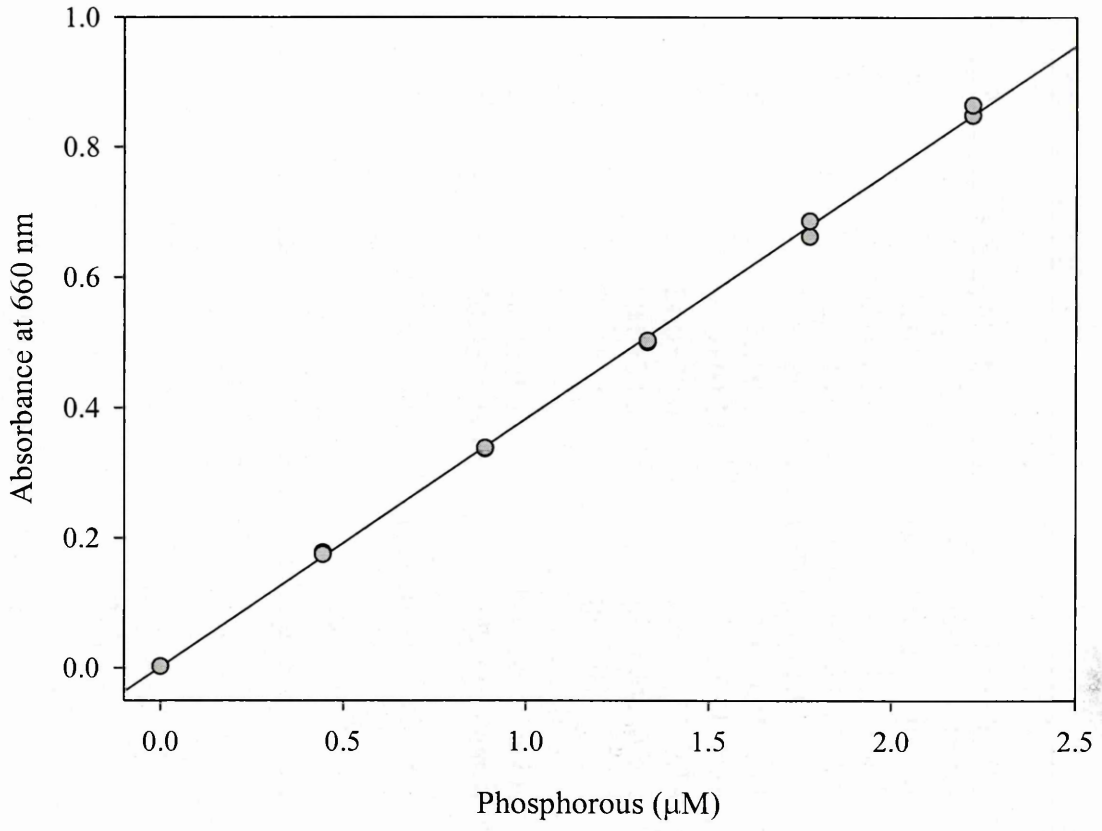

Figure 3.20. An example of a typical calibration curve for the determination of phosphorus using potassium dihydrogen orthophosphate as a standard. $R^2 = 99.9\%$.

Table 3.23. Summary of phosphorous content data for *Euphausia superba* available in the literature. Measurements on whole animals unless otherwise stated. All data expressed as dry mass. (SE, standard error; *n* = total number of krill analysed; * ± 95% confidence limits).

Study	Analysis method	Antarctic location	Time of year	Krill analysed	Phosphorus ± SE	*n*
Ikeda (1984)	Molybdate method after digestion in 50% H_2SO_4 at 100 °C (Strickland & Parsons, 1972)	Off Wilkes Land	Jan	Juvenile (10–50 mg)	1.23±0.62*	3
				Juvenile (50–100 mg)	0.89±0.22*	4
				Adult (100–200 mg)	0.75±0.24*	4
				Adult (200–300 mg)	0.81±0.25*	5
				Gravid females (>300 mg)	0.78±0.17*	3
Ishii et al. (1987)	Digested in 50% H_2SO_4, neutralised with KOH and phosphorus content measured as phosphate	—	Summer	11.2–19.6 mg	1.02±0.05	15
				28.4–49.7 mg	1.04±0.10	6
				81.6–112.1 mg	0.97±0.12	7
				105.6–269.8 mg	0.88±0.07	9
				173.4–360.0 mg	1.03±0.07	16
Ikeda and Kirkwood (1989)	Molybdate method after digestion in 50% H_2SO_4 at 100 °C (Strickland & Parsons, 1972)	Off Enderby Land	Nov	Juvenile (< 50 mg)	1.18±0.02	21
				Juvenile (50–100 mg)	1.19±0.01	29
				Adult (100–200 mg)	1.17±0.03	14
Ikeda and Dixon (1982)	Molybdate method after digestion in 50% H_2SO_4 at 100 °C (Strickland & Parsons, 1972)	101–115 E 64 S	Jan	Juvenile (10–100 mg)	1.03±0.041	38

Results

C, H and N

For JR70 data alone the carbon values had a mean of 48.4% with a range of 40.2% to 52.4% and a CV of 6.51%. For hydrogen the mean was 7.44%, with a range of 6.53% to 8.07% and a CV of 5.56%. Nitrogen had a mean of 9.47%, with a range of 8.08% to 11.50% and a CV of 10.10%. There was no significant relationship with length for any of the elemental components, or with frozen mass.

There was a significant relationship between sexual maturity stage and carbon content (ANOVA, $F_{7, 40} = 6.79$, $p < 0.001$) and to a lesser degree with pooled sex (ANOVA, $F_{4, 43} = 2.96$, $p = 0.030$). Similarly, nitrogen showed significant differences between sexual maturity stages (ANOVA, $F_{7, 40} = 8.79$, $p < 0.001$) and to a lesser degree with pooled sex (ANOVA, $F_{4, 43} = 3.49$, $p = 0.015$). Hydrogen also showed a similar trend, with the difference between sexual maturity stages (ANOVA, $F_{7, 40} = 5.70$, $p < 0.001$) being greater than between pooled sexes (ANOVA, $F_{4, 43} = 3.45$, $p = 0.016$). Carbon to nitrogen ratio showed a significant difference between sexual maturity stages (ANOVA, $F_{7, 40} = 5.84$, $p < 0.001$), but not with pooled maturity stages (Table 3.1).

There was no difference between swarms or sampling events for any of the elements, nor with on- or off-shelf locations or chlorophyll content, although there was a significant difference between the chlorophyll composition of on- and off-shelf stations (ANOVA, $F_{1, 46} = 4.71$, $p = 0.035$).

Table 3.24. Elemental composition of Antarctic krill (% dry mass) from JR70. (SE, standard error; *n* = total number of krill analysed). Data in italics is shown for pooled sexual maturity classifications (adult females, sub-adult and adult males).

	Carbon		Hydrogen		Nitrogen				
	Mean	SE	Mean	SE	Mean	SE	C : N	*n*	Length (mm)
All	48.4	0.45	7.44	0.06	9.47	0.14	5.19	48	34–62
J	47.6	0.70	7.31	0.10	9.45	0.24	5.06	7	34–44
MS1	49.0	0.92	7.52	0.13	9.24	0.32	5.37	8	36–45
MS2	49.3	1.11	7.62	0.16	8.91	0.27	5.58	7	41–47
MS	*49.1*	*0.69*	*7.56*	*0.10*	*9.08*	*0.21*	*5.47*	*15*	*36-47*
MA1	51.2	1.01	7.71	0.15	8.50	0.24	6.04	3	55–56
MA2	42.2	0.73	6.67	0.07	11.32	0.07	3.73	5	52–56
MA	*45.6*	*1.73*	*7.06*	*0.20*	*10.26*	*0.53*	*4.60*	*8*	*52–56*
FS	48.9	0.82	7.49	0.11	9.10	0.21	5.43	11	35–46
FA3	48.9	—	7.47	—	9.68	—	5.05	1	53
FA4	50.4	0.33	7.71	0.06	10.02	0.13	5.03	6	51–62
FA	*50.1*	*0.35*	*7.68*	*0.06*	*9.97*	*0.12*	*5.03*	*7*	*51–62*

To enable a better comparison between JR70 and JR82, samples from the growth rate study were pooled with the above data as there was no difference between means (*t*-test for elements, $p > 0.05$ in all cases). For all data pooled (Both JR70 cruises and JR82), the carbon values had a mean of 46.5% with a range of 37.6% to 53.3% with a CV of 8.09%. For hydrogen there was a mean of 7.10%, with a range of 5.81% to 8.34% and a CV of 9.73%. For nitrogen there was a mean of 9.66%, with a range of 7.87% to 11.50% and a CV of 8.48% (Table 3.25).

Table 3.25. A comparison between the elemental composition of Antarctic krill sampled from the Scotia Sea on consecutive years. (SE, standard error; *n* = total number of krill analysed). Data in italics is shown for pooled sexual maturity classifications (adult females, sub-adult and adult males).

	JR70								JR82							
	Carbon		Hydrogen		Nitrogen				Carbon		Hydrogen		Nitrogen			
	Mean	SE	Mean	SE	Mean	SE	*n*	Length(mm)	Mean	SE	Mean	SE	Mean	SE	*n*	Length (mm)
All	48.2	0.22	7.37	0.04	9.32	0.06	158	31–62	44.2	0.32	6.76	0.05	10.08	0.06	127	21–60
J	47.4	0.49	7.26	0.08	9.57	0.18	11	31–44	44.5	0.48	6.81	0.07	10.02	0.09	57	21–44
FS	48.5	0.37	7.37	0.06	9.20	0.11	50	34–46	50.7	—	7.84	—	8.34	—	1	42
FA1	47.7	—	7.46	—	10.03	—	1	49	42.7	0.55	6.51	0.07	10.44	0.09	33	33–54
FA2	—	—	—	—	—	—	0	—	43.6	1.12	6.68	0.13	10.34	0.05	3	47–52
FA3	47.0	1.92	7.24	0.19	9.90	0.19	3	53–57	44.0	1.52	6.77	0.24	10.17	0.51	3	49–56
FA4	50.2	0.40	7.59	0.09	10.08	0.10	9	51–62	48.5	0.73	7.43	0.11	9.84	0.09	13	49–58
FA5	45.4	0.64	7.02	0.01	9.23	0.56	3	53–54	42.3	0.90	6.43	0.13	9.41	0.11	10	49–60
FA	*48.6*	*0.64*	*7.41*	*0.08*	*9.89*	*0.14*	*16*	*49–62*	*44.0*	*0.47*	*6.71*	*0.07*	*10.13*	*0.08*	*62*	*33–60*
MS1	47.9	0.40	7.30	0.07	9.27	0.10	45	31–45	43.0	—	6.52	—	10.70	—	1	33
MS2	49.2	0.49	7.56	0.09	8.98	0.12	26	35–53	46.9	—	7.05	—	9.60	—	1	48
MS3	52.9	—	7.82	—	8.13	—	1	55	—	—	—	—	—	—	0	—
MS	*48.4*	*0.32*	*7.40*	*0.06*	*9.15*	*0.08*	*72*	*31–55*	*44.9*	*1.98*	*6.78*	*0.26*	*10.15*	*0.55*	*2*	*33–48*
MA1	51.2	1.01	7.71	0.15	8.50	0.24	3	55–56	44.4	0.48	6.80	0.12	9.93	0.07	3	45–47
MA2	43.1	1.03	6.80	0.15	10.88	0.45	6	51–56	41.2	0.99	6.38	0.13	10.82	0.33	3	48–56
MA	*45.8*	*1.53*	*7.11*	*0.18*	*10.08*	*0.50*	*9*	*51–56*	*42.5*	*0.96*	*6.55*	*0.13*	*10.46*	*0.28*	*5*	*45–56*

For pooled JR70 data, the carbon values had a mean of 48.2% with a range of 40.2% to 53.3% with a CV of 5.8%. For hydrogen there was a mean of 7.37%, with a range of 6.27% to 8.34% and a CV of 5.96%. Nitrogen had a mean of 9.32%, with a range of 8.08% to 11.50% and a CV of 8.48%. JR82 data was as follows; carbon values had a mean of 44.2% with a range of 37.6% to 53.0% with a CV of 7.61%. For hydrogen there was a mean of 6.76%, with a range of 5.81% to 8.10% and a CV of 7.78%. Nitrogen had a mean of 10.08%, with a range of 7.87% to 11.32% and a CV of 6.40%.

There was a significant positive relationship between carbon and length for JR70 pooled data ($r^2 = 6.6\%, p = 0.001$,) and for JR82 animals ($r^2 = 4.1\%, p = 0.022$). Hydrogen also showed a significant positive relationship with length for both cruises respectively ($r^2 = 7.8\%, p < 0.001$); ($r^2 = 3.6\%, p = 0.032$). Nitrogen was negatively correlated with length for krill from JR70 ($r^2 = 5.1\%, p = 0.011$), but showed no relationship in animals sampled during JR82.

When the data were compared between sexual maturity stages, there was a significant difference in the JR70 pooled data for carbon (ANOVA, $F_{10, 147} = 5.14$, $p < 0.001$), nitrogen (ANOVA, $F_{10, 147} = 6.26$, $p < 0.001$) and hydrogen (ANOVA, $F_{10, 147} = 2.69$, $p = 0.005$). In JR82 the same is true, with carbon (ANOVA, $F_{10, 116} = 4.02$, $p < 0.001$), nitrogen (ANOVA, $F_{10, 116} = 4.81$, $p < 0.001$) and hydrogen (ANOVA, $F_{10, 116} = 5.28$, $p < 0.001$). However, when pooled sex classes were considered, there was no difference with carbon or hydrogen, although nitrogen remains highly significant; JR70 (ANOVA, $F_{4, 153} = 6.27$, $p < 0.001$), JR82 (ANOVA, $F_{4, 122} = 2.60$, $p = 0.039$) (Table 3.25 and Figure 3.21, Figure 3.22 and Figure 3.23).

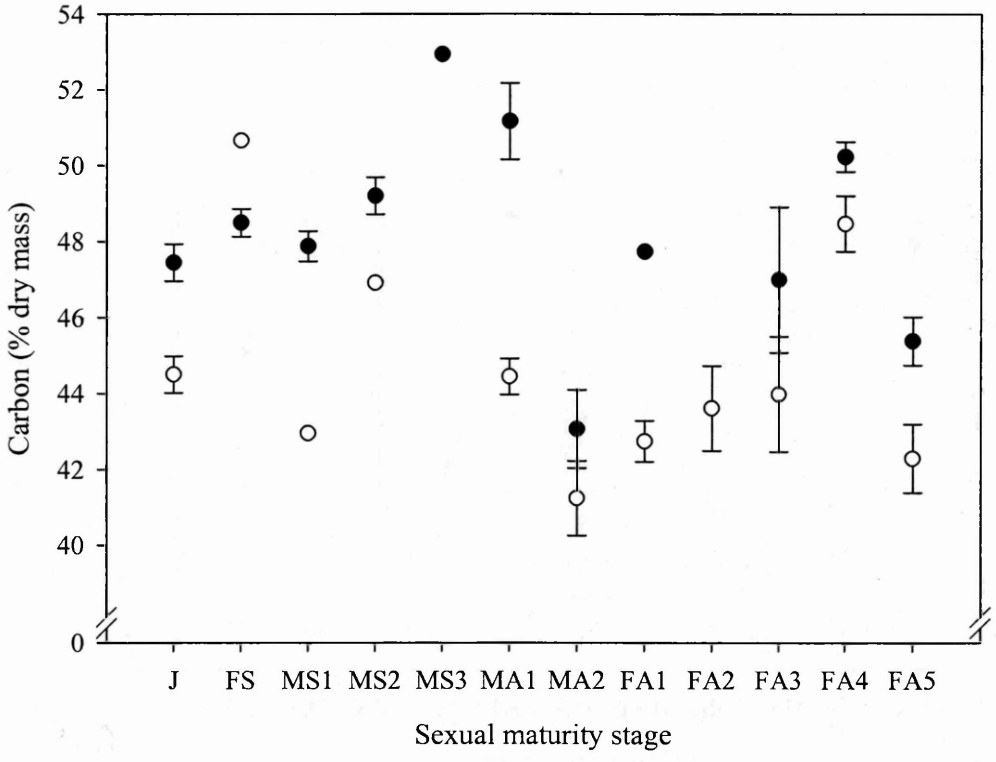

Figure 3.21. Differences in total carbon content (% of dry mass) between sexual maturity stages in krill from JR70 (filled circles) and JR82 (open circles). Values are means ± 1 standard error.

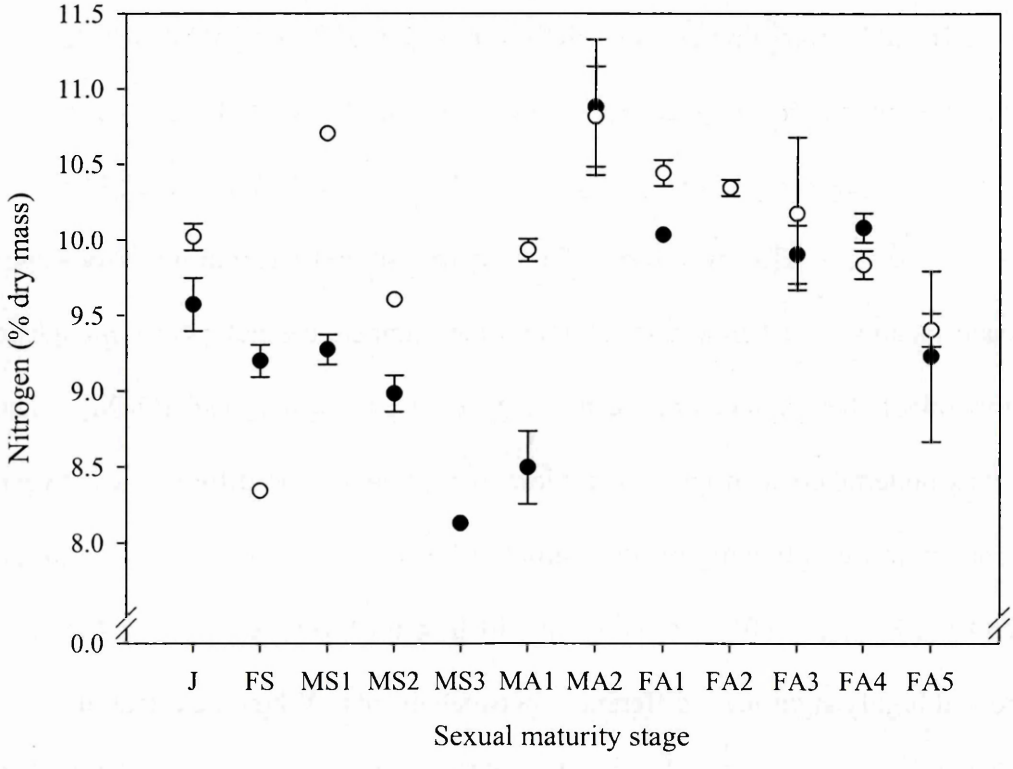

Figure 3.22. Differences in total nitrogen content (% of dry mass) between sexual maturity stages in krill from JR70 (filled circles) and JR82 (open circles). Values are means ± 1 standard error.

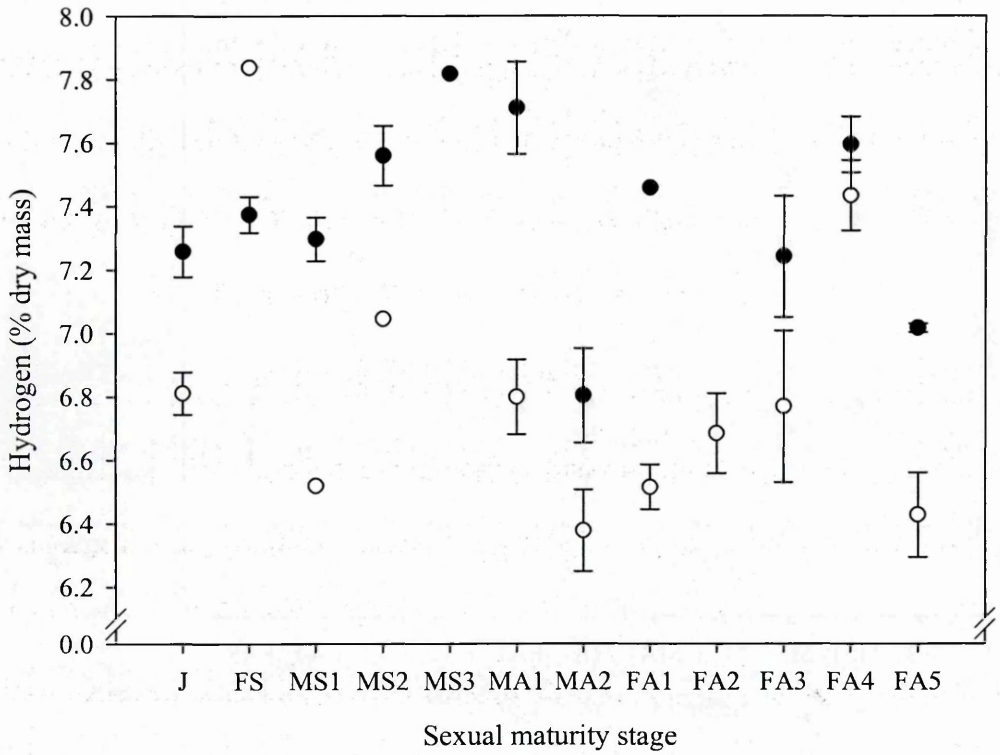

Figure 3.23. Differences in total hydrogen content (% of dry mass) between sexual maturity stages in krill from JR70 (filled circles) and JR82 (open circles). Values are means ± 1 standard error..

For the JR70 samples there is a significant difference with swarm (ANOVA, $F_{25, 132} = 2.03$, $p = 0.005$) and by sampling event (ANOVA, $F_{17, 140} = 2.28$, $p = 0.005$) with carbon, nitrogen; (swarm (ANOVA, $F_{25, 132} = 3.03$, $p < 0.001$), event (ANOVA, $F_{17, 140} = 2.96$, $p < 0.001$)) and hydrogen (swarm (ANOVA, $F_{25, 132} = 2.99$, $p < 0.001$) and event (ANOVA, $F_{17, 140} = 3.49$, $p < 0.001$)). However, the differing composition of sexual maturity stages sampled at each location is a factor here, although the numbers are not robust enough to allow examination of these interactions using a generalised linear model (GLM). The cruise JR82 was undertaken with more of a view to elucidating the differences between sampling events, with the following results; carbon: (ANOVA, $F_{23, 103} = 4.96$, $p < 0.001$), nitrogen (ANOVA, $F_{23, 103} = 6.01$, $p < 0.001$) and hydrogen (ANOVA, $F_{23, 103} = 4.09$, $p < 0.001$) all showed highly significant differences between events. When the effect of sex is removed, and only one sex at a time is considered, the results are more meaningful: with juvenile krill, there is a significant relationship between events with carbon, (ANOVA, $F_{18,}$

$_{38} = 5.69, p < 0.001$), nitrogen (ANOVA, $F_{18, 38} = 4.18, p < 0.001$) and hydrogen (ANOVA, $F_{18, 38} = 5.74, p < 0.001$). When the same analysis is done for FA1 animals there is significant difference with carbon (ANOVA, $F_{14, 18} = 7.56, p < 0.001$), nitrogen (ANOVA, $F_{14, 18} = 5.46, p = 0.001$) and hydrogen (ANOVA, $F_{14, 18} = 3.73, p = 0.005$), although 4 of these events included only one animal; when these events were removed, only carbon remains significantly different (ANOVA, $F_{10, 18} = 3.42, p = 0.011$).

When environmental differences were considered for JR82 krill, there was a significant relationship between longitude and carbon content for all krill pooled (r^2 = 31.7%, $p < 0.001$), with carbon increasing from west to east. Similarly carbon increased significantly with latitude, although this was less pronounced (r^2 = 14.3%, $p < 0.001$), increasing from south to north. When only juvenile krill were considered, the relationship with latitude was less significant (r^2 = 20.0%, $p < 0.001$), but showed a more marked increase from south to north. The relationship with longitude again existed with a trend for greater carbon content in the more easterly samples (r^2 = 52.6%, $p < 0.001$) which was more marked and of a greater magnitude than that for all krill pooled. For FA1, there was a reduced range of longitudes sampled, but there was still a significant relationship (r^2 = 30.3%, $p = 0.001$); similarly with latitude ($r^2 = 41.1\%, p < 0.001$). This trend was likely to be related to chlorophyll concentrations; as chlorophyll is significantly correlated with longitude and latitude, and carbon composition strongly correlates with chlorophyll at sampling station (r^2 = 14.2%, $p < 0.001$), where an increase in chlorophyll is related to high carbon composition. This pattern holds for juvenile krill alone, but is not significant for FA1 animals. However, there is a much stronger relationship with C : N ratio (an indicator of condition) and chlorophyll ($r^2 = 22.9\%, p < 0.001$).

By comparison, krill from JR70 showed no difference between animals caught on- and off-shelf the continental shelf and their C : N ratio, carbon or hydrogen content when there was no significant difference in chlorophyll concentration between on- and off-shelf

sampling locations. Nitrogen content was, however, higher on- compared to off-shelf (ANOVA, $F_{1, 156} = 4.75, p = 0.031$).

Phosphorus

Phosphorus content in the krill sampled ranged from 1.14% to 1.46%, with a mean of 1.29% and a CV of 5.24%. There was no relationship between phosphorus composition and length or dry mass. However, there was a small, but not significant difference between sexes (ANOVA, $F_{10, 103} = 1.90, p = 0.053$), but not with pooled sexes (Table 3.26, Figure 3.24). It is interesting to note, that although there was no significant difference between sexual maturity stages, FA4 and FA5 individuals had a markedly higher phosphorus content, likely to be related to the cellular change associated with rapid growth of the ovaries.

There was also no significant difference between the phosphorus composition of krill from different swarms, or sampling locations. When analysed by chlorophyll content at sampling station and on and off-shelf sampling location, no difference in phosphorus composition was found.

Table 3.26. Phosphorus content of Antarctic krill. (SE, standard error; *n* = total number of krill analysed). Data in italics is shown for pooled sexual maturity classifications (adult females, sub-adult and adult males).

	Phosphorous (% dry mass)			
	Mean	SE	*n*	Length (mm)
All	1.29	0.01	114	31-58
J	1.26	0.03	4	31–39
MS1	1.29	0.01	37	31–45
MS2	1.28	0.02	21	35–53
MS3	1.22	—	1	55
MS	*1.28*	*0.01*	*59*	*31–55*
MA2	1.17	—	1	51
MA	*1.17*	—	*1*	*51*
FS	1.30	0.01	39	34–45
FA1	1.16	—	1	49
FA2	1.19	—	1	unknown
FA3	1.25	0.05	3	53–57
FA4	1.34	0.05	3	54–58
FA5	1.35	0.05	3	53–54
FA	*1.29*	*0.03*	*11*	*53–58*

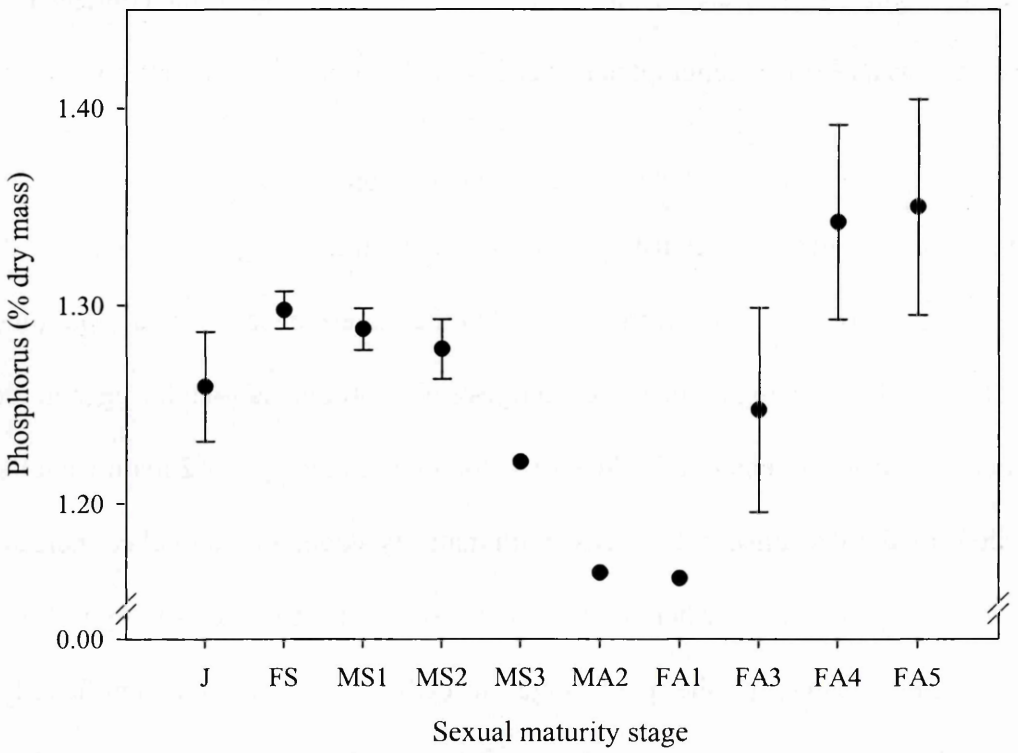

Figure 3.24. Difference in total phosphorus content (% of dry mass) between sexual maturity stages in krill from JR70. Values are means ± 1 standard error.

Discussion

Elemental composition reflects the variability seen with proximate composition of krill, discussed above. Overall, variation in elemental composition was lowest in phosphorus with carbon, hydrogen and nitrogen showing inconsistent levels of variance between differing cruises. For all data pooled, hydrogen was the most variable element, then nitrogen then carbon. Hydrogen was similarly the most variable component in JR82, with carbon showing the next highest variability then nitrogen. For JR70 pooled data, nitrogen was the most variable, then hydrogen and carbon. The composition of sexual maturity stages sampled was different between the cruises, with krill encountered during JR82 typically having few fully sexually mature individuals. If there are patterns in elemental composition with maturity and sampling location, this could explain the differing variability in elements between cruises. This could be attributable to an assumed difference in proximate composition of the krill from different cruises. We have already hypothesised that lipid levels were lower in krill from JR82 (using water content as a proxy), potentially as a result of geographical changes in chlorophyll concentration.

Variation with sexual maturity stage

There were significant relationships with sexual maturity stages for all elements except phosphorus. Carbon content strongly reflected the pattern observed with lipid (and carbohydrate); typically showing an increase from juvenile, through sub-adult stages in the male krill, then a significant depletion in the spermatophore producing MA2 mature adults. Mature female krill show a consistent increase with maturity stage, with a marked increase at FA4, the heavily gravid stage, when a significant proportion of the total body is filled with oocytes. Post spawning, the percentage of carbon was reduced significantly, representing the loss of the lipid rich eggs. Nitrogen is essentially the mirror image of the patterns seen with carbon, where an apparent decrease in nitrogen results from the increase in carbon mass, where as hydrogen follows the general pattern of carbon. It is interesting

that, although protein is the most dominant fraction of the krill's dry mass, variation in the elemental composition reflects that of lipid more strongly. This is because lipid is approximately 70% carbon, so any changes in lipid will reflect strongly in the elemental composition. (See Chapter 5 for further discussion). For phosphorus composition, there was no significant difference between sexual maturity stages, although FA4 and FA5 animals have a much higher level of phosphorus, which could result from higher RNA concentrations resulting from the rapid growth of the ovaries and egg production.

Morphometric variation

A significant positive relationship between length and carbon composition was found in krill collected during both cruises, a relationship which has been demonstrated by several previous studies (Ikeda, 1984; Ishii et al., 1987; Huntley et al., 1994). It is likely that this relationship is influenced by the larger lipid rich mature krill, particularly the females. However, when each sexual maturity stage is considered separately (where data is available), juvenile and sub-adult male and female krill show a significant positive relationship with carbon and length, the mature female stages show a weak negative relationship. Hydrogen reflects the relationships shown with carbon, whilst nitrogen shows a negative relationship. It is likely that this increase with carbon with body length reflects in increased storage of lipid as krill mature.

Effects of environmental variability

For JR70 animals there was a significant difference in C, H and N between swarm and sampling event, although there was no relationship with chlorophyll concentration of surface water at the sampling station or the on- or off-shelf location. Carbon, hydrogen and nitrogen all showed significant differences with sampling event during JR82, with an increase in carbon and hydrogen in krill from sampling events with higher chlorophyll concentrations and a decrease in nitrogen. It is intuitive that elemental composition should correlate with food availability, as we have previously seen how the proximate components

are affected by changes in chlorophyll, and how elemental composition is related to this. However, no studies have been undertaken to explicitly examine this link between elemental composition and food availability in krill.

Spatial and temporal variability

In krill from JR82, the relationship with sampling event could be due to the significant relationship between longitude and carbon content, with carbon increasing from east to west. Also carbon increased with latitude, increasing from south to north. This relationship is still apparent when individual sexual maturity stages are considered alone. As described previously, chlorophyll is correlated with latitude and longitude, and it likely to be this that is driving the changes in chemical composition. Again, no studies have examined the effect of spatial variability on the elemental composition of krill.

When the two sampling years are compared, there is a highly significant difference with all elements, but when krill sampled in the same geographical area are compared, there is no significant difference in their elemental composition.

Overall, sampling limitations (specifically the number of animals and analysis time) meant that it was not possible to analyse the chemical composition data in such a way that would allow for the complex interactions between physiological, environmental and temporal sources of variation to be teased apart.

Data in this chapter are also published in: Arnold, K. H., Shreeve, R. S., Atkinson, A., & Clarke, A., 2004. Growth rates of Antarctic krill, *Euphausia superba*: Comparison of the instantaneous growth rate method with nitrogen and phosphorus stoichiometry. *Limnology and Oceanography*, **49**, 2152-2161.

Chapter 4 Variation with growth rate

These trends in the chemical make up of krill, and the changes in composition with the life cycle described in the previous chapter indicate that detailed investigations of aspects of chemical composition can produce insights in to the physiology and ecology of organisms. During JR70, fellow workers (Angus Atkinson, Rachael Shreeve and Geraint Tarling) were conducting an experiment to assess the growth rates of krill (subsequently published as Pond et al., 2005; Shreeve et al., 2005; Atkinson et al., 2006; Tarling et al., 2006), so the opportunity was taken to examine aspects of the chemical composition of these animals in relation to their known growth rates.

Central to any understanding of population dynamics is a knowledge of growth rate. Much data on krill growth have been gathered (e.g., Mackintosh, 1972a; Buchholz et al., 1989; Ross et al., 2000), yet spatial and temporal variability in growth is poorly understood (Quetin et al., 1994). There are inherent difficulties in studying the growth of crustaceans, which retain no skeletal record of past growth. A variety of approaches have been used to investigate krill growth, including assessments of the length frequency of natural populations (Mackintosh, 1972a), those eaten by predators (Reid et al., 2002) and direct measurements with instantaneous growth rate (IGR) method (Ross et al., 2000).

Each of these approaches has its strengths and weaknesses: field studies measuring growth directly with the IGR method yield invaluable results (Nicol et al., 1992b; Ross et al., 2000), but are time consuming and many krill need to be incubated individually. Changes in length frequency distributions in krill populations have been used to estimate

growth rates, either through direct sampling (Rosenberg et al., 1986; Miller & Hampton, 1989), or through the diet of predators (Reid et al., 2002). However, the results can be equivocal, relying on the assumptions that the same population is being sampled over time and that mortality is not size dependent. To improve our knowledge of growth rate responses in krill, we thus need alternative methods that are fast and efficient, in order to examine variability in growth over a wide range of scales.

Biochemical indicators of growth are one such possible alternative. For many organisms including krill, the ribonucleic acid (RNA) content or its ratio to deoxyribonucleic acid (DNA) has been found to vary with growth rate (Sutcliffe, 1970; Båmstedt & Skjoldal, 1980; Ikeda, 1989). More recently, studies of freshwater crustaceans have suggested a relationship between elemental composition (most notably phosphorus) and growth rate (Main et al., 1997; Elser et al., 2000a). Most phosphorus in zooplankton is bound in ribosomal RNA (rRNA) (Main et al., 1997), and organisms with high rRNA exhibit high maximum growth rates. This suggests a consistent positive association between growth rate and RNA, and hence phosphorus.

The growth rate hypothesis (Elser et al., 2000b) states that *"differences in organismal C:N : P ratios are caused by differential allocations to RNA necessary to meet the protein synthesis demands of rapid rates of biomass growth and development"* (Sterner & Elser, 2002). The prediction emerging from this hypothesis is that a negative relationship exists between molar N: P ratios and specific growth rates in organisms, driven by differences in rRNA, the machinery of protein synthesis required for growth. To date there have been few direct tests of this theory (Main et al., 1997; Elser et al., 2000a). Most tests of the hypothesis have, to date, focused at an interspecific level on freshwater zooplankton, with little work on whether such a relationship can be demonstrated on an intraspecific (between individual) basis. The only study to date that has considered intraspecific relationships (Main et al., 1997) demonstrated that there was a significant

relationship between N : P and growth within individuals in only one of the three species of *Daphnia* studied.

Using chemical composition data for *Euphausia superba,* the first test of the *growth rate hypothesis* using a marine zooplankton species was undertaken. The hypothesis was tested on two levels:

- Intraspecifically, whether the N : P ratio of individual krill showed a relationship with their growth rate during the previous moult cycle (approximately two weeks), as measured with the instantaneous growth rate technique. That is, the N : P ratio will vary on the same spatial and temporal scales as observed growth rate.
- Interspecifically whether the relationship described for freshwater zooplankton species between N : P and growth rate also applies to *E. superba.*

MATERIALS AND METHODS

Experimental design

The instantaneous growth rate (IGR) method measures linear growth increments of krill maintained in laboratory incubations, at the ambient temperatures encountered at the point of sample collection (Quetin & Ross, 1991). The method assumes that growth increments (the percentage increase or decrease in krill length at the moult) measured during the first few days of incubation represent growth rates in the field (Nicol et al., 1992b). Elemental analysis was conducted on individual krill whose growth was known: each individual selected for analysis had moulted within 5 days of capture, and thus had a known growth increment derived from shipboard IGR experiments.

Field methods

Krill were sampled during JR70, as described in Chapter 2, which presents a good field example with which to test the growth rate hypothesis, with samples collected within a limited area with no seasonal effects. To ensure experimental krill were in good

physiological condition, haul duration was no longer than 20 minutes and a solid cod end was used. Freshly caught krill were transferred immediately to 150 litre circular bins containing seawater at ambient temperature. Healthy krill were then transferred individually to 300 ml perforated containers and held in tanks of free-flowing (1 litre $minute^{-1}$) filtered seawater maintained at ambient temperature. Krill were incubated in this way aboard ship for five days, and checked daily for moulting. For all individuals that had moulted, total length, length of uropod of the animal and the exuviae, and the sex and maturity stage were determined according to Makarov and Denys (1981). To reduce measurement error, all measurements were carried out by Rachael Shreeve aboard ship under low power magnification (x12) fitted with an ocular micrometer. These individuals were then frozen at -80°C and biochemical analysis conducted in the UK using the methods described in Chapter 3.

Growth rate

Individual growth rates were determined using the Instantaneous Growth Rate (IGR) method (Quetin & Ross, 1991; Ross et al., 2000). The fraction of krill that moulted during the incubations was recorded, and for each swarm the intermoult period (IMP) calculated from the total number of individuals moulted in the experiments (N_{moult}), the total number incubated ($N_{incubated}$) and the duration of the experiments (t) using the following equation:

Equation 4.1

$$IMP = \frac{(t \, x \, N_{incubated})}{N_{moult}}$$

Dead or dying krill and those that moulted during the course of the incubations were removed from the tanks, but were still included in the total number incubated ($N_{incubated}$).

The difference in the length of the uropods of moulted exoskeletons and the post-moult animals was measured using an ocular micrometer, to determine growth (or shrinkage) per intermoult period as a percentage of the initial length. Total length of the post-moult krill (LT_1) (mm) and the growth increment determined from the uropod measurements (GI) (%) were used to estimate the length of the krill at the beginning of the incubation (LT_0) (mm) using the following equation:

Equation 4.2

$$LT_0 = LT_1\left[\frac{(100-GI)}{100}\right]$$

Lengths at T_0 were converted to dry mass (DMT_0) using the equation:

Equation 4.3

$$DMT_0 = 0.00000106\ LT_0^{3.15}$$

Equation 4.3 is as given in Appendix 3 of Morris et al. (1988), for a relationship derived for all experimental krill, anterior-telson measurement. This equation was used as the number of moulted animals in this study were too few to derive a precise regression from our data alone. Dry mass at T_1 (DMT_1) was as described in Chapter 3. It is worth noting here that krill 'shrinkage' or negative growth can be measured by this method and occurs when a negative GI is encountered, i.e. the uropod of the moulted krill is smaller than that of its exuvium.

Specific growth rate (SGR) for each krill was then estimated as:

Equation 4.4

$$SGR = \frac{[\ln(DMT_1) - \ln(DMT_0)]}{IMP}$$

Statistics

All data were checked for normality using the Anderson-Darling normality test and found not to deviate significantly from normal. The contributions of the growth increment

(GI) and intermoult period (IMP) to variance in specific growth rate (SGR) were estimated as:

Equation 4.5

$$Var[SGR] \approx SGR^2 \left[\left(\frac{Var[IMP]}{IMP^2} \right) + \left(\frac{Var[GI]}{GI^2} \right) - 2 \left(\frac{Cov[IMP, GI]}{IMP \, x \, GI} \right) \right]$$

where SGR, IMP and GI are the mean values for the variables, Var[SGR], Var[IMP] and Var[GI] are the variances and Cov[IMP,GI] is the covariance between growth increment and intermoult period (all values calculated from the pooled means from each swarm).

RESULTS

Instantaneous growth rate

Specific growth rate ranged from -0.010 to 0.041 g dry mass day^{-1}, with a coefficient of variation (CV) of 80%. Krill from separate swarms displayed significantly different mean growth rates. (ANOVA $F_{14, 95}=9.91$, $p<0.001$) (Table 4.1, Table 4.2). Growth rate was measured in krill sampled between 7 January and 31 January 2002. There was a general decrease in growth rate through the month (Figure 4.1). The variation in specific growth rate comprises both changes in the intermoult period and the variation in individual growth increments. Plots of both IMP and growth increment as a function of sampling event indicate that variation in krill growth rate is driven mainly by changes in growth increment (Figure 4.2), which contributes ~ 91% of the variation in specific growth rate. As might be predicted, a negative relationship was found between length and specific growth rate ($F_{1, 108}=5.78$, $p=0.018$, $r^2=5.1\%$). There was no significant relationship between growth rate and sexual maturity stage of the animals (ANOVA $F_{4, 105}=1.74$, $p=0.147$).

Table 4.1. Summary of results by swarm. Growth rate is specific growth rate (g d^{-1}). Statistics are shown for one-way ANOVA by swarm. (SE: standard error, n = total number of krill).

Sampling	Length (mm)		Specific growth rate		Growth increment (%)			
event	Mean	Range	Mean	Range	Mean	Range	IMP	n
32.2	40.50	40–41	0.009	0.006–0.012	3.90	2.49– 5.31	14	2
33.1	38.50	31–49	0.018	0.002–0.041	8.15	0.75–17.67	15	20
89.1	41.86	37–55	0.017	0.005–0.028	7.52	2.53–12.50	15	28
89.2	39.71	35–43	0.013	0.005–0.016	8.67	3.28–11.00	23	7
134.2	54.00	53–55	0.007	0.003–0.010	4.33	2.05– 6.18	20	3
164.1	39.60	36–49	0.005	-0.001–0.010	3.54	-0.88– 7.27	24	5
165.1	40.00	—	0.008	—	4.28	—	18	1
165.2	40.67	38–43	0.002	0.000–0.006	1.63	0.00– 4.89	25	3
170.1	37.00	33–43	0.002	-0.003–0.006	1.17	-1.70– 3.12	16	6
171.1	35.33	33–38	-0.003	-0.010–0.002	-1.41	-5.26– 1.02	17	3
252.2	46.58	31–58	0.005	0.000–0.020	2.78	0.00– 10.98	18	19
254.1	41.00	—	0.003	—	2.39	—	23	1
254.2	46.00	43–49	0.003	0.003–0.003	1.50	1.39– 1.60	17	2
276.2	41.67	37–47	0.006	0.000–0.011	2.90	0.00– 5.08	15	9
302.1	41.00	—	0.000	—	0.00	—	20	1

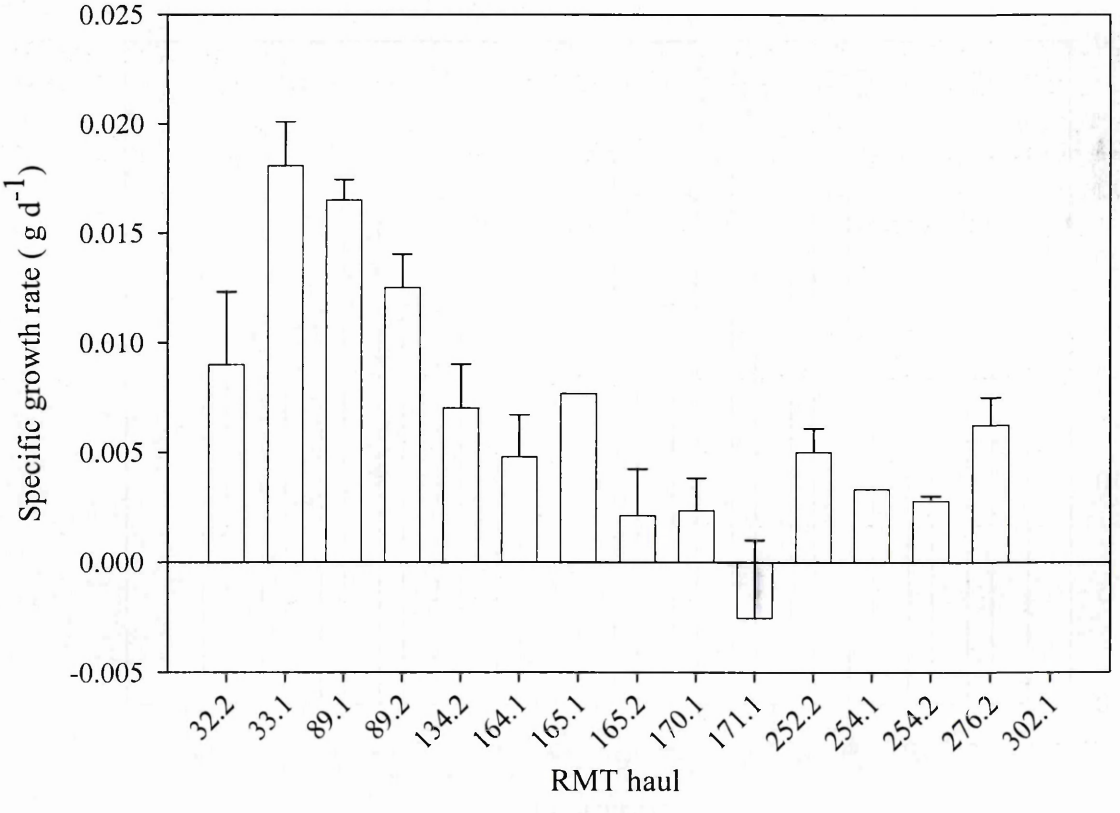

Figure 4.1. Differences in specific daily growth rate (g d^{-1}) with sampling event. Data are means for separate swarms. RMT hauls increment with time and cover swarms sampled from 7–31 January 2002. Error bars display one standard error (no error bars shown for n<2). The number of moulters measured for each haul ranged from 1 to 28 (median 5). Note that haul 302.1 has n=1, SGR = 0.0.

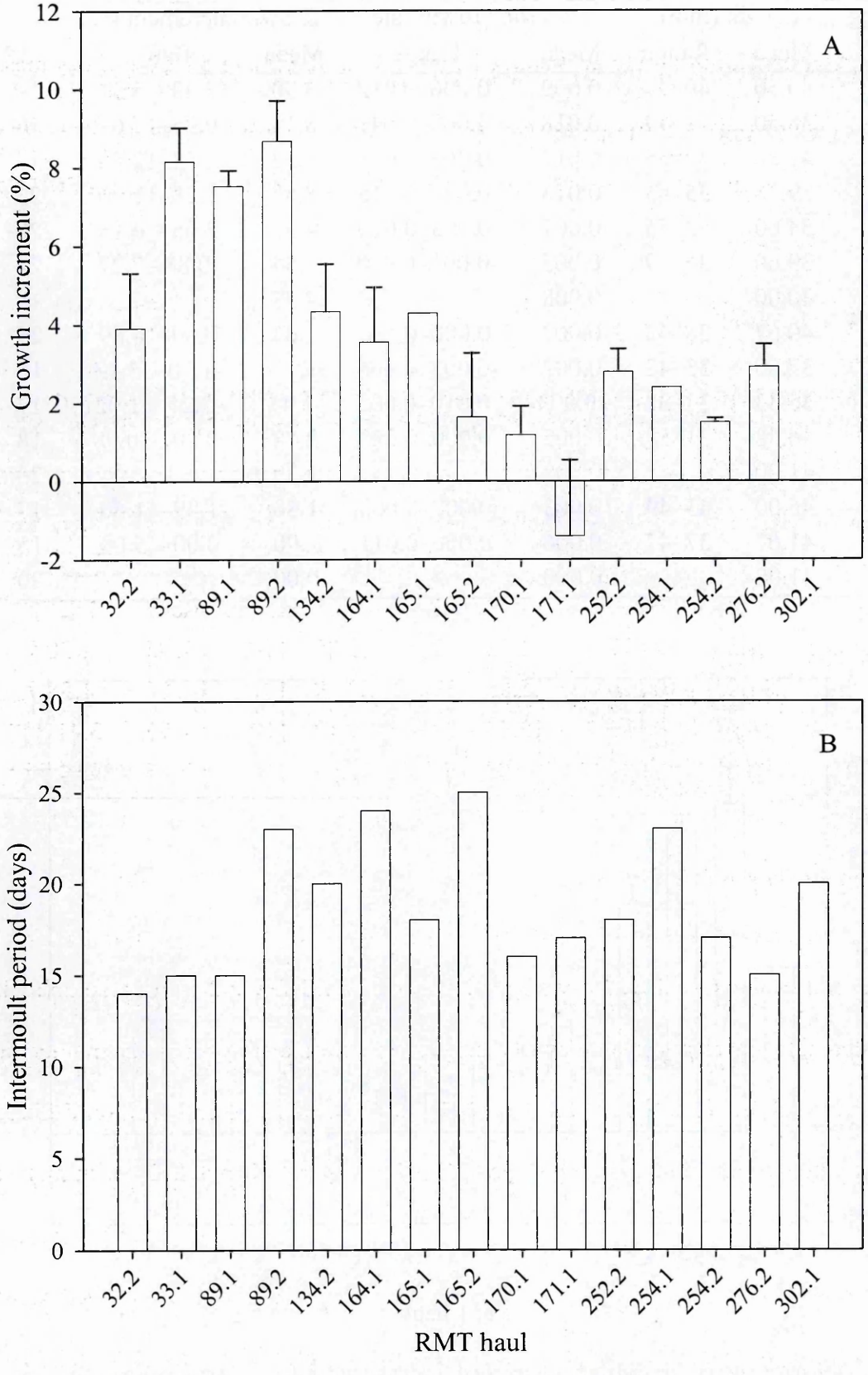

Figure 4.2. Variation of (A) growth increment, and (B) intermoult period between swarms. Error bars shown in A display standard error (intermoult period is a mean value for each swarm).

Chemical composition

Overall data

Carbon content of all the krill studied varied from 42.13% to 53.25% of dry mass, N from 8.13% to 11.36%, P from 1.13% to 1.44% and mineral ash from 5.05% to 19.84% (Table 4.2). Inorganic C was very low, ranging from 0% to 1.99%, with a mean of 0.26%. Less variation was observed in the phosphorus composition of krill than with nitrogen (CV P%=5.3%, N%=7.6%).

Table 4.2. Elemental and mineral ash composition (% dry mass) and growth rate (g d^{-1}) of *Euphausia superba*, for all individuals. Statistics are shown for one-way ANOVA by swarm. (SE: standard error, CV: coefficient of variation, n = total number of krill).

	Mean	SE	range	CV%	n	df	F
Carbon	48.16	0.249	42.13 – 53.25	5.42	110	14, 95	2.70 **
Hydrogen	7.33	0.043	6.27 – 8.34	6.09	110	14, 95	4.31 ***
Nitrogen	9.26	0.067	8.13 – 11.36	7.58	110	14, 95	4.92 ***
Phosphorus	1.28	0.007	1.13 – 1.44	5.30	110	14, 95	1.30 †
Ash	11.23	0.226	5.05 – 19.84	23.79	101	14, 86	1.38 †
N : P	16.04	0.139	13.57 – 20.61	9.10	110	14, 95	3.41 ***
Growth rate	0.01	0.001	-0.01 – 0.04	79.64	110	14, 95	9.91 ***

*** $p<0.001$
** $p<0.005$
† not significant

Variation with sexual maturity stage

Several of the sexual maturity stage classes contained few individuals, so animals were pooled into coarser sexual maturity stage categories; disregarding the numerical maturity stage subdivisions (i.e., J, FS, MS, MA and FA). Only N composition differed significantly between sex and maturity stage (ANOVA $F_{4, 105}=2.57$, $p=0.042$), with mature females displaying the highest %N, mature males the lowest. C and H content varied relatively little with maturity stage, but the data for mineral ash content showed some variation (not significant), with higher values for mature classifications (FA and MA) (Table 4.3).

Table 4.3. Elemental and mineral ash composition (all data % dry mass) of Antarctic krill, *Euphausia superba* shown by grouped sexual maturity stage (stages according to Makarov & Denys, 1981 with MS including MS1-3, MA including MA1-2 and FA including FA1-5). (SE: standard error, *n*=total number of krill).

	% Carbon		% Nitrogen		% Hydrogen		% Phosphorus		% Ash		
Stage	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*n*
J	47.22	0.67	9.78	0.22	7.16	0.12	1.23	0.04	10.43	0.70	4
FS	48.36	0.41	9.23	0.12	7.34	0.07	1.29	0.01	11.03	0.35	39
MS	48.24	0.36	9.17	0.08	7.35	0.07	1.28	0.01	11.05	0.39	57
MA	47.28	*	8.65	*	7.50	*	1.16	*	14.40	*	1
FA	47.32	0.94	9.82	0.23	7.20	0.09	1.30	0.04	13.18	1.40	9

Variation with size

Within the size range of krill sampled (31–58 mm), there were weak relationships between chemical composition and length (Figure 4.3). Where relationships were statistically significant (C, N, ash), r^2 values were small (C%: $F_{1,\ 108}$=15.68, p<0.001, r^2=12.7%; N%: $F_{1,\ 108}$=4.66, p=0.033, r^2=4.1%; and ash: $F_{1,\ 99}$=5.60, p=0.020, r^2=5.4%). Elemental composition was recalculated as a fraction of the ash-free dry mass (AFDM), with carbon expressed as organic C (C in mineral ash subtracted from total C); these organic data are summarized in Table 4.4. There was a stronger correlation between organic carbon and total length ($F_{1,\ 98}$=44.15, p<0.001, r^2=31.1%), but the relationship between nitrogen and phosphorus as a percentage of AFDM was not significant.

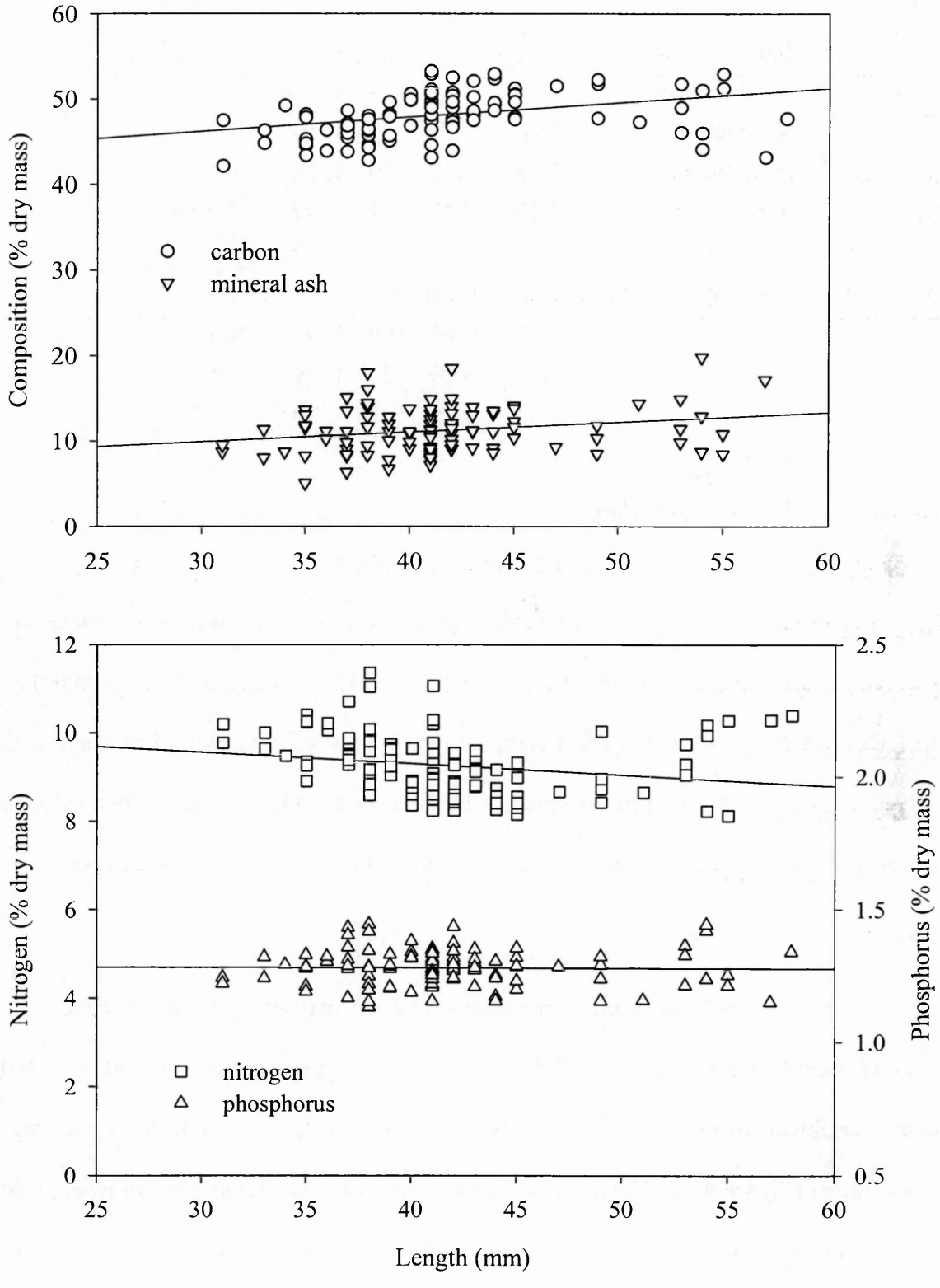

Figure 4.3. Relationship between elemental and mineral ash composition (% dry mass) and total length. Regressions significant for C% ($F_{1, 108}$=15.68, p=<0.001, R^2=12.7%), N% ($F_{1, 108}$=4.66, p=0.033, R^2=4.1%) and ash ($F_{1, 99}$=5.60, p=0.020, R^2=5.4%) but not P% ($F_{1, 108}$=0.02, p=0.889, R^2=0%).

Table 4.4. Elemental composition of Antarctic krill, *Euphausia superba*. Statistics shown are for regression analysis with total length. (SE: standard error, *n*=total number of krill).

Component	Mean	SE	*n*	*df*	*F*
Dry mass basis (% dry mass)					
Total carbon	48.16	0.25	110	1, 108	15.68 ***
Nitrogen	9.26	0.07	110	1, 108	4.66 *
Phosphorus	1.28	0.01	110	1, 108	0.02 †
Ash	11.23	0.27	101	1, 99	5.60 *
Organic basis (% ash-free dry mass)					
Organic carbon	53.87	0.27	100	1, 98	44.15 ***
Nitrogen	10.42	0.08	101	1, 99	2.56 †
Phosphorus	1.44	0.01	101	1, 99	0.38 †

*** $p<0.001$
* $p<0.05$
† not significant

Variation with swarm

When chemical data were analysed between swarms, significant differences were observed in %N (ANOVA $F_{14, 95}=4.92$, $p<0.001$), %C (ANOVA $F_{14, 95}=2.70$, $p<0.005$) and %H (ANOVA $F_{14, 95}=4.31$, $p<0.001$) composition. The possibility existed to examine the differences between two swarms sampled at the same location in 3 instances, but it was felt that sample sizes in these swarms were insufficient to make any meaningful comparisons.

Variation with growth rate

There were no significant relationships between percentage N, P, or molar N : P ratio and growth rate over all the individuals pooled by regression analysis (n=110, p>0.05 for all variables), even when the data are sorted by length (smaller krill having larger growth rates) (Figure 4.4). However, the swarms had different mean growth rates (Table 4.1 and Table 4.2) so the data were also analysed after pooling by swarm. When this was done there was a significant variation in %N ($F_{14,95}=4.92$, $p<0.001$) and N : P ($F_{14,95}=3.41$, $p<0.001$) between swarms (Figure 4.5), but there was no significant relationship between N : P ratio or N% dry mass and specific growth rate when data were pooled by swarm and analysed by regression analysis. Data were also analysed for relationships between growth

rate and elemental composition within swarms, but significant relationships were found in only two cases; event 164.1 (P%: $F_{1,3}$=44.82, p=0.007, r^2=93.7%) and event 170.1 (N : P: $F_{1,4}$=10.01, p=0.034, r^2=71.4%), although the number of individuals was small in both cases.

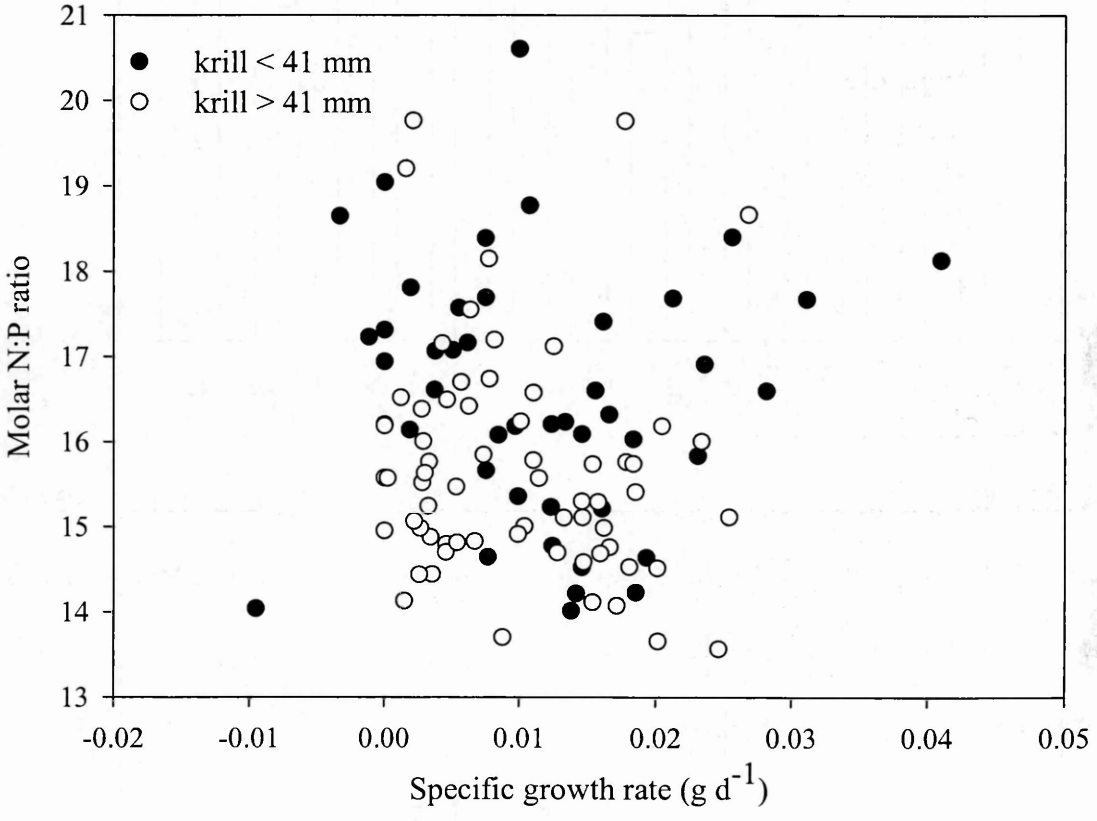

Figure 4.4. Relationship between specific growth rate and N: P ratio in *Euphausia superba*. Closed circles are small krill (< 41 mm), open circles are large krill (≥ 41 mm)

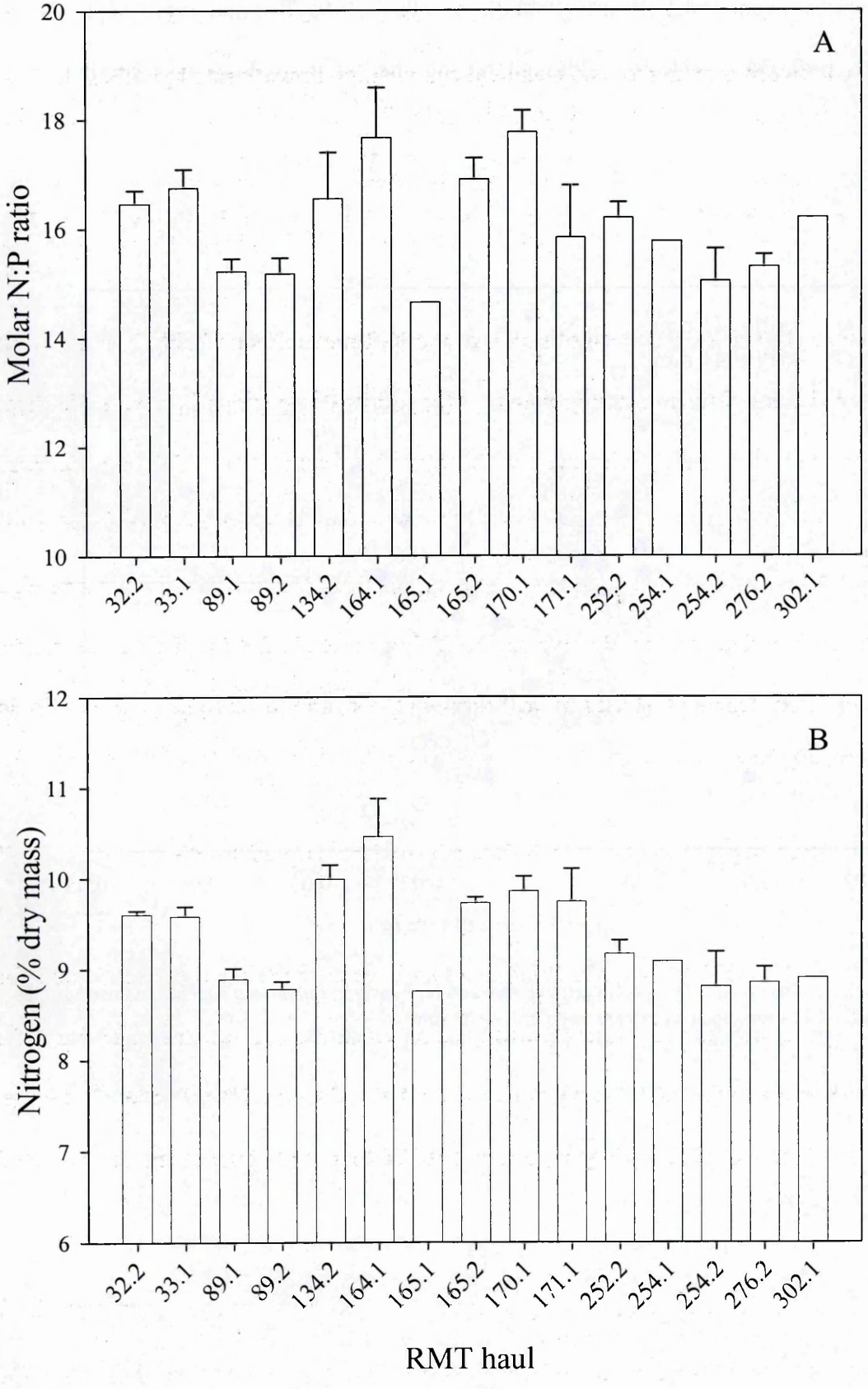

Figure 4.5. Differences in (A) molar N: P ratio, and (B) N content (% dry mass) between swarms. Error bars display standard error.

DISCUSSION

This section has two main threads; krill growth rate is discussed first, and then the relationship between growth rate and chemical composition (growth rate hypothesis).

Growth rate measured by the IGR method

This study is only the fifth study to measure krill growth directly with the IGR method, even though this is acknowledged as the best and most direct method available (Nicol, 2000). The relative under-utilisation of the IGR method is probably because of its labour-intensive and time-consuming nature. Our results demonstrate how variable these observed growth rates are in krill, even within a single swarm. Individual krill showed great variability, from negative growth to rapid growth, within just a small scale of space and time. Of interest are the negative growth rates recorded in some individuals in high summer, even in a famously productive region (Atkinson et al., 2001). This could reflect either the different feeding histories of krill forming the swarm, or individual variability in feeding ability within a swarm.

This study represents the first IGR study of krill around South Georgia. Krill growth varied markedly from individual to individual: In summer and in the same general area, krill growth varied from slight shrinkage to values near the maximum recorded for krill of this size (Nicol, 2000). Our growth results (which are equivalent to 0.07 mm d^{-1}) are near the maximum GIs of 4.4% (Quetin & Ross, 1991, fall), 15.7% (Nicol et al., 1992b, summer), 6% (Nicol et al., 2000b, summer) and 10% (Ross et al., 2000, summer) reported by the 4 previous IGR studies.

Three other studies using techniques other than IGR have previously reported krill growth from the South Georgia region; (Clarke & Morris, 1983) (0.33 mm d^{-1}), (Rosenberg et al., 1986) (0.148 mm d^{-1}) and (Reid, 2001) (0.130 mm d^{-1}). These studies have each employed different techniques to measure growth rate, and the discrepancy between them

shows that these methods could be prone to error. Clarke and Morris (1983) reported growth rates three times faster than any other published value (Quetin et al., 1994), and Reid (2001) suggested that krill in his study did not grow at all during January (compare with our observed January growth rates, Figure 4.1).

A significant correlation was observed between growth rate and sampling event (and hence date). The majority of this variation is attributable to growth increment at moult rather than moulting frequency, although IMP did vary between swarms. It is possible that the local environmental conditions at each sampling location, such as water temperature and food quality and availability, were triggering different rates of growth. Surface chlorophyll *a* values encountered during the cruise ranged from 0.19–18.67 mg m^{-3}. Provisional analysis with hourly chlorophyll samples and CTD data from the cruise show a weak but statistically significant positive correlation with growth (Atkinson et al., 2006). However, it is difficult to relate observed growth to environmental variability, and such work is beyond the scope of this chapter. The apparent seasonal decline in growth rate evident in Figure 4.1 cannot reliably be ascribed to temporal variation, as there are many other variables, such as chlorophyll *a* concentrations, spatial distribution and water temperature, which may have also influenced the growth rates observed. This variability in observed growth highlights the need for a better understanding of the causes of this variation and the development of a suitable proxy for growth to help us achieve this.

Chemical composition

Overall data

The chemical composition of Antarctic krill determined in this study is similar to that reported previously (Ishii et al., 1987; Huntley et al., 1994; Torres et al., 1994). In contrast to previous stoichiometry studies on other zooplankton (Main et al., 1997; Elser et al., 2000a) there was less observed variation in the phosphorus composition of krill than with nitrogen. It is possible that the higher variation in N composition could have resulted

from differing food conditions where the krill were sampled. Studies of vascular plants (Shaver & Melillo, 1984) and chlorophytes (Rhee, 1978) have shown a significant relationship between their N composition and nutrient supply, although comparable studies on invertebrate zooplankton suggest that they tend to maintain relatively stable stoichiometric composition (Andersen & Hessen, 1991; Demott et al., 1998), even after different food manipulations in the laboratory (Hessen, 1990).

Deposition of lipid commensurate with growth affects the C:N ratios of zooplankton (Sterner & Elser, 2002) but this accumulation of C is unlikely to affect directly the N : P ratio, although it may dilute its component measurements (P and N when measured as a function of dry mass). Although phosphorus is an important component of phospholipids, these typically contribute less than 5% of total body mass in most organisms (Sterner & Elser, 2002) and therefore do not explain whole animal patterns in a way that can be seen with RNA. In Antarctic krill, between 7 and 40% of total phosphorus is attributable to phospholipids. The highest proportions are confined to females with mature ovaries, as eggs contain large amounts of phospholipid in the egg yolk; in juveniles and males the majority of P is contributed by RNA (BAS, unpublished data) (see also Chapter 5).

Variation with sexual maturity stage

Although the krill incubated represented a range of sexual maturity stages, most of those that moulted were sub-adult females and males, which has resulted in the unbalanced nature of the sexual maturity stage categories in this study, and the need to pool sex and maturity stages for statistical analysis. Only %N varied significantly with sexual maturity stage. The significant relationship between %N and sexual maturity stage is probably attributed to the low value for the mature male and high values for mature females contributing to most of the variation. This difference is driven by the large size of the

ovary in gravid females (FA3 and FA4), rich in C and N (lipid and vitellogenin in yolk) but relatively low in P, despite the phospholipid component of yolk.

Variation with size

Weak relationships were shown between length and C, N and ash composition. We would expect little variation in chemical composition with size of the animals when the results are expressed as percentage composition, as the animals are likely to have a broadly similar physiological make up as they grow (Ikeda, 1984). There are, however, life history reasons for some amount of variability such as reproductive stage and season; thus the observed change in ash content with length could be related to a size-dependent change in the proportion of the exoskeleton. However, we can see that differences in composition can be attributed to sexual maturity stage and there is naturally an element of the size of the animal inherent in this distinction as the two factors co-vary. The observed variation in elemental composition with size and maturity stage are, however, small.

Variation with growth rate

Between swarms there was a significant difference in %N and N : P (Figure 4.5), with the difference in N : P driven principally by variation in N and not P (as discussed above). No relationship could be detected between specific growth rate and either %N, %P or N : P whether analysed between events or for all data pooled.

Intraspecific test of the growth rate hypothesis

Our results thus suggest that Antarctic krill at South Georgia in late summer do not support the growth rate hypothesis; there is no relationship between N : P ratio and growth rate when comparisons are made between individuals or when data are pooled by swarm. The most likely explanation for this is the lack of variation in phosphorus content. As all the krill were sampled during the summer in a period of maximum growth, it seems likely that they are all physiologically prepared to exploit the patches of good food quality and

quantity with periods of rapid growth. As krill do not grow in length continuously, but instead grow in bursts dictated by the moult cycle, any particular individual krill may have shown no growth during the duration of the experiment, but may demonstrate positive growth on its previous and potentially on its next moult. Furthermore, during this summer period it is unlikely that animals would alter the cellular concentration of ribosomes over short time-scales, as it would be both costly to reduce this and then re-synthesize ribosomes when conditions allow a period of high growth. Zooplankton typically live in a variable environment where food is patchy, and they must be capable of exploiting food whenever they find it. To do so, they maintain their capacity for growth (high ribosomal content) for the summer period when they are likely to meet short-term concentrations of food. I speculate therefore, that the N: P ratio may more accurately be described as reflecting potential growth rate; where a krill with a low N : P ratio has the potential to display high growth rates (apparatus for growth ready), although growth rate in the previous IMP has been low.

It was predicted, however, that a comparison between summer and winter krill from the same area would reveal variations in %P which could be attributed to long-term (seasonal) changes in cellular rRNA which could be correlated to a reduced growth rate observed during winter months. (It is hypothesised that krill in the winter have a high N : P ratio as they have a reduced potential for growth). This prediction is supported by recent data from Antarctic krill sampled from Deception Island, where RNA:DNA ratio and citrate synthase activity were lower in krill sampled in early spring (after over-wintering) than in high summer (Cullen et al., 2003).

It was not possible to obtain any krill sampled in the winter from the same area studied here; however winter krill caught in June from the Bransfield Strait were kindly supplied by Dr Robin Ross of the Palmer Long Term Ecological Research programme. These animals had been used in oxygen consumption and ammonia excretion experiments,

then freeze dried. Sex and maturity stages were assigned as follows; immature, male or female and length recorded using the standard measure (S1). In order to compare lengths with the AT measurement used by this study, a conversion was applied using the equation of Siegel (1987). Chemical analysis was carried out as described previously, dry mass and fresh mass data was as supplied by Dr Ross. A summary of the results of the analysis of the winter krill is presented in Table 4.5.

Despite significant differences in N (ANOVA $F_{1,\ 170}=5.78$, $p=0.017$) and P (ANOVA $F_{1,\ 170}=8.31$, $p=0.004$) content between the summer and winter krill, there was no significant difference between the N : P ratio between the krill from the summer and winter. Although phosphorus content was significantly lower in the winter as predicted, nitrogen was also significantly reduced so that the ratios remained unchanged. This contradicts our theory. It is possible that winter conditions were favourable that year and the animals were still growing, but we can only hypothesise as no direct growth rate measurements were taken. Alternatively, the data could have been influenced by the smaller size of many of the krill from the winter sample; however it was shown that there was a negative relationship in these krill between nitrogen content and length, so a bias towards smaller krill is unlikely to have artificially lowered the nitrogen concentration.

Table 4.5. Winter chemical composition data from krill sampled in the southern Bransfield Strait in June All data % dry mass, except water, % fresh mass (SE, standard error; n = total number of krill analysed). * Ash n= 60 for all data, n = 20 for females. Length is derived from measured S1 length, converted to AT length using the equation of Siegel (1987).

	Carbon		Hydrogen		Nitrogen			Phosphorus			Mineral ash		Water			Length
Stage	Mean	SE	Mean	SE	Mean	SE	C : N	Mean	SE	N : P	Mean	SE	Mean	SE	n	(mm)
All	49.0	0.28	7.10	0.04	9.00	0.09	5.49	1.25	0.01	7.25	9.70	0.24	78.0	0.30	62*	26–58
Immature	48.3	0.39	6.99	0.06	9.14	0.11	5.31	1.24	0.02	7.37	8.66	0.29	78.2	0.31	22*	26–38
Male	49.9	0.42	7.23	0.08	8.71	0.13	5.76	1.28	0.02	6.81	10.12	0.28	77.5	0.57	20	34–58
Female	48.9	0.60	7.09	0.09	9.14	0.18	5.41	1.23	0.02	7.43	10.31	0.54	78.4	0.65	20	27–52

Interspecific test of the growth rate hypothesis

Although a relationship between growth rate and N: P could not be demonstrated for krill on an individual or swarm level, it is possible that the overall mean matches the growth rate hypothesis. To ensure comparability of data for this analysis, specific growth rate (SGR) data for krill have been recalculated to conform with the definition used by Main et al. (1997), namely

Equation 4.6

$$SGR = 0.5\left[\ln\left(\frac{B_{final}}{B_{initial}}\right)\right]$$

where B_{final} is the final biomass and $B_{initial}$ the initial biomass

When the mean data for *Euphausia superba* from this study (all samples combined) were compared with the data for freshwater zooplankton (Main et al., 1997), the data from this study fit the general interspecific relationship between elemental stoichiometry and growth rate previously demonstrated (Figure 4.6). The krill data from this study fall just within the 95% confidence levels for the overall regression, with a closer fit than data for *Bosmina longirostris*. This would suggest that at a population level, late summer *Euphausia superba* at South Georgia are in line with the overall data for freshwater zooplankton. However it should be noted that the data in Figure 4.6 mix several populations of some species, with an overall mean for Antarctic krill.

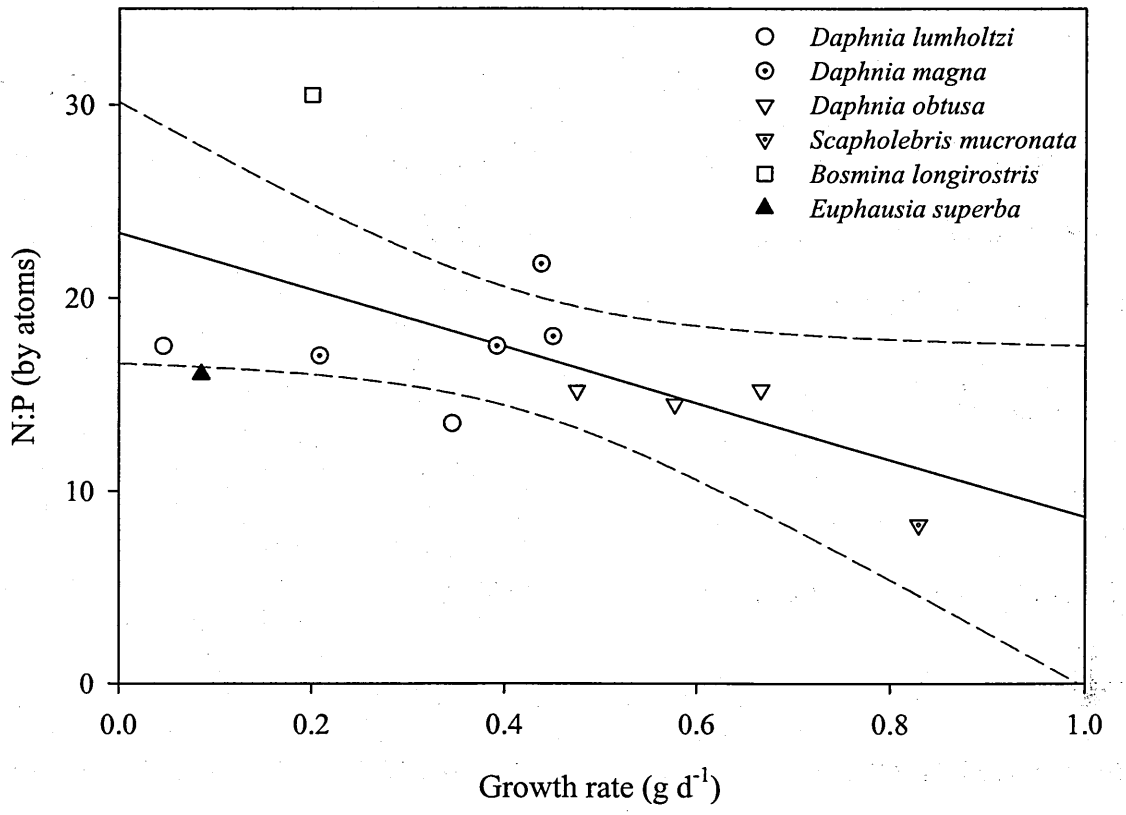

Figure 4.6. Relationship between N : P ratio and growth rate from Main et al. (1997), with data from this study for *Euphausia superba* plotted. Solid line shows Main's regression, dashed lines 95% confidence limits.

Despite the wealth of literature on theoretical support for the '*growth rate hypothesis*', (Sterner & Elser, 2002 and references therein) there exist few practical demonstrations of its application outside of the field of freshwater zooplankton. In this light the broad agreement observed on a larger scale suggests that further investigation of marine invertebrates will yield positive results. Comparisons of N and P content of summer and winter krill would therefore be of interest because on a seasonal timescale we might expect the very large changes in growth rate to be reflected in the chemistry, although the analysis will be complicated by concomitant changes in body mass. In doing so, however, care needs to be taken to allow for the physiology of growth: short-term variations in growth rate may not be related to elemental composition. This means that elemental composition cannot provide a proxy for short-term variations in growth rate. It remains important, therefore, to pursue the discovery of a suitable biochemical proxy for

growth rate in krill and to further test the suitability of this technique. Once such potential proxy for short-term growth rates is the development of a technique using diatom fatty acids as an indicator of growth (Pond et al., 2005).

From this study we conclude that N and P stoichiometry do not provide a useful proxy for recent growth rate in *Euphausia superba* on an intraspecific basis, or over short time scales. However, if the data for summer krill are pooled to provide a long-term average for elemental composition and growth rate, the data for all individuals in this study are broadly comparable with the interspecific growth rate relationship demonstrated by Main et al. (Main et al., 1997) for 5 freshwater crustaceans (Figure 4.6). This would suggest that when averaged over a period of a month, the population of krill at South Georgia do match the growth rate hypothesis and lends some practical support to the idea that this hypothesis is a general relationship that can be applied over size, species and habitat boundaries, as implied by stoichiometric theory.

Chapter 5 A stoichiometrically consistent composition for Antarctic krill

INTRODUCTION

The previous chapters (Chapters 3 and 4) have demonstrated the importance of understanding the biochemical composition of krill with respect to their life-history and environmental variability. Many fundamental studies of species ecology and ecosystem functioning rely on an understanding of the relationships between the biochemical composition of organisms and their environment. Because of this need for data on chemical composition for studies of ecosystem function and the interaction of species within it, it is imperative that we have reliable ways of collecting such data and of maximising the information to be gained from these data. As discussed previously (Chapter 4) elemental composition data have many practical advantages over proximate composition determination: only small amounts of sample are required to obtain complete elemental composition (C, H, N and P), replicates can be analysed for the same individual krill and small zooplankton species can be analysed individually without the need for pooling of samples. However, for many applications, it is desirable to have the resolution and insight provided by proximate biochemical composition analysis, especially in respect to the major components of growth, reproduction and storage – protein, lipid and carbohydrate.

This chapter aims to examine whether it is possible to reliably predict the proximate biochemical composition of krill, using only elemental composition data. In a seminal paper Gnaiger and Bitterlich (1984) proposed a theoretical approach to this, which they tested using the soft tissues of fish and molluscs. This method has subsequently been applied to cnidarians (Zamer et al., 1989), freshwater crustacean zooplankton (Riccardi & Mangoni, 1999; Riccardi, 2000) and eight marine gelatinous zooplankton from the

Southern Ocean (two ctenophores, two siphonophores, an anthomedusan, a scyphozoan, an annelid and a salp) (Clarke et al., 1992).

In this chapter, the proximate and elemental composition data from Chapter 3 are used to examine:

- How well the analysis of separate proximate components on individual krill explains the overall biochemical composition of krill at varying life history stages.
- If this biochemical composition reflects the elemental composition derived for the same life history stages.

The potential for deriving an accurate proximate biochemical composition for krill from elemental composition data is investigated and its applications and limitations discussed. Here we are asking the question:

- Can a stoichiometrically consistent composition for Antarctic krill, *Euphausia superba* be derived?

Most researchers concentrate on either elemental or proximate composition; rarely are the two compared in a stoichiometrically consistent manner.

STOICHIOMETRIC ANALYSIS

Proximate composition

In studies of the biochemical composition of marine invertebrate tissues, researchers generally have been unable to account for the entire organic mass of their samples as the sum of protein, carbohydrate and lipid mass fractions (Madin et al., 1981; Zamer et al., 1989). Typically the discrepancy results from not including small molecular mass metabolites, nucleic acids and any significant structural components such as chitin in crustaceans. However, results from this study, as shown in Table 5.1, demonstrate that close to 100% of total body composition was accounted for by the proximate determination of water, mineral ash, protein, lipid, carbohydrate, chitin and nucleic acid fractions. For all

life-history stages investigated this agreement was broadly consistent, although it is clear from Table 5.1 that there is a large amount of variance in the data presented, resulting from a combination of the accumulation of individual variability and experimental sensitivity. Variance has been used in preference to standard error in this case as it is possible to sum the variances of different analysis to asses the variance associated with the total (Rothery, pers. comm). There is no demonstrable increase in accuracy gained from pooling life-history stages, with these categories not improving the explanation of total mass, or appreciably reducing variability.

Table 5.1. The sum of all the proximate biochemical fractions analysed for various life-history stages of Antarctic krill. Data presented as % fresh mass, Var = variance, CHO = carbohydrate.

		Water	Ash	Protein	CHO	Lipid	Chitin	RNA	DNA	Total
All	Mean	74.38	2.89	11.97	0.39	6.42	1.86	0.29	0.1	98.29
	Var	4.37	0.11	0.48	0.01	4.58	0.91	0.01	0.01	10.46
J	Mean	74.95	3.05	11.85	0.37	6.16	1.24	0.28	0.11	98.01
	Var	3.8	0.04	0.88	0.01	3.49	0.17	0	0	8.38
MS1	Mean	73.67	2.88	11.68	0.43	6.278	1.75	0.33	0.11	97.13
	Var	2.13	0.02	0.24	0.01	3.98	0.58	0	0	6.96
MS2	Mean	73.68	2.87	11.57	0.2	8.09	2.52	0.23	0.09	99.25
	Var	3.84	0.03	0.26	0	0.27	1.57	0	0	5.98
MS3	Mean	72.03	2.83	11.57	0.3	7.42	3.62	0.27	0.09	98.14
	Var	—	—	0	0	1.56	0.1	0	0	1.66
MS	Mean	73.62	2.88	11.62	0.35	7.1	2.22	0.28	0.1	98.17
	Var	2.68	0.02	0.18	0.01	2.82	1.16	0.01	0	6.89
MA1	Mean	73.33	2.93	11.85	0.37	9.81	2.54	0.2	0.08	101.11
	Var	0.99	0	0.12	0	3.22	0.31	0	0	4.65
MA2	Mean	77.58	3.31	12.39	0.4	2.57	1.69	0.2	0.09	98.22
	Var	2.04	0	0.41	0.01	0.66	1.1	0	0	4.23
MA	Mean	76.25	3.21	12.19	0.39	4.18	2.01	0.2	0.09	98.51
	Var	5.76	0.03	0.35	0.01	11.1	0.91	0	0	18.16
FS	Mean	74.44	2.97	11.78	0.39	7.3	1.91	0.29	0.11	99.18
	Var	4.8	0.02	0.31	0	1.15	1.25	0	0	7.54
FA3	Mean	73.8	2.53	11.61	—	7.23	1.54	0.45	0.1	97.25
	Var	0.02	—	—	—	—	—	—	—	0.02
FA4	Mean	73.25	2.23	12.83	0.51	6.35	1.37	0.42	0.08	97.04
	Var	0.76	0.1	0.26	0.02	0.25	0.06	0.02	0	1.46
FA	Mean	73.33	2.27	12.66	0.51	6.47	1.4	0.42	0.08	97.14
	Var	0.68	0.09	0.43	0.019	0.318	0.06	0.02	0	1.62

Proximate and elemental comparison

In order to be able to say whether the data obtained by various measures of proximate composition are consistent with the results of the elemental analysis, we must break each proximate component down into its elemental composition. This was done by considering the structural chemistry of each component in turn, with the molecular compositions ascribed to each component displayed in Table 5.6 and described as follows:

Water

Krill water content can be simply estimated as all moisture lost on drying, ascribed to pure water (H_20). Some water is retained in oven-dried tissue (Gnaiger & Bitterlich, 1984) but usually this error is small and affects only the hydrogen content significantly.

Chitin

The structural polymer chitin was described here as a linear homopolymer of N-acetyl-β-D-glucosamine, with the molecular mass described as that of the subunit, less one hydrogen lost on polymerisation.

Inorganic elemental component of mineral ash

The inorganic components of animals may potentially be a significant component of their total biomass, especially in animals with large skeletons or shells (Sterner & Elser, 2002). However, krill have comparatively little in terms of hard skeleton, their exoskeleton being composed principally of chitin (Marquardt & Carreno, 1992). Despite this, potentially significant amounts of inorganic elements may be found as minerals complexed in the chitin matrix, or present in other parts of the krill's body.

The amount of inorganic and residual organic carbon, hydrogen and nitrogen remaining in the mineral ash was assessed by CHN analysis of triplicate (where possible) mineral ash samples using the procedure described in Chapter 3, with the mean values presented in Table 5.6.

Carbohydrate

Carbohydrates include a small amount of mono and di-saccharides involved as metabolites together with polysaccharides such as starch and glycogen; differing structural polymers based on the glucose monomer. Because chitin has previously been considered and is not measured in the carbohydrate assay, we can make the assumption that all carbohydrate in krill is glycogen, with the structural formula based on a repeating glucose monomer with the loss of two hydrogen and one oxygen molecule representing the loss of a water molecule on polymerisation.

Protein

Proteins are polymers comprised of various sequences of around 20 different amino acids. The number, composition and sequence of amino acids determine the biochemical properties of each specific protein. Amino acids vary in chemical composition, for example N content ranges from ~ 8% in tyrosine to 35% in arginine (see Table 5.2). If the 20 amino acids were equally represented, the elemental composition of the average protein would be ~ 53% C, 17% N and 0% P (Sterner & Elser, 2002).

In reality, amino acids are not evenly distributed in animal proteins; rather each species has a distinct pattern of amino acid composition. Fortunately, the amino acid composition of *Euphausia superba* has been well studied as a result of numerous investigations into its use as a potential resource for human consumption and as an aquaculture feed. Table 5.3 presents a summary of the range of published amino acid profiles for krill protein. This balanced amino acid composition does indeed make krill suitable for use in both human and aquaculture food supplements (Mauchline, 1980a).

To decide upon the 'typical' amino acid composition for krill protein, an average of the amino acid compositions from all the studies was considered, however, the range in methodologies and sexual maturity stages of the krill analysed, and the season and location

at which they were collected could potentially introduce a considerable amount of variability. Instead, the figures of Ferguson and Raymont (1974) for krill caught near South Georgia in January (Table 5.3) were used because they are directly comparable with the krill analysed for biochemical and elemental composition in this study. The sum of all determined amino acids by Ferguson and Raymont was 99.8%, so the individual totals were adjusted to give a total of 100%. From this amino acid profile, a theoretical stoichiometric formula for 'standard krill protein' was calculated using the molecular composition by mass described in Table 5.2.

Table 5.2. Carbon, nitrogen, hydrogen, oxygen and sulphur composition (molecular and percentage of total mass) of the amino acids. This list includes only those amino acids reported in acid hydrolysates of *Euphausia superba* from South Georgia in January, as described by Ferguson and Raymont (1974).

	Molecular formula					Molecular	% by mass				
	C	H	O	N	S	mass	C	H	O	N	S
Alanine	3	7	2	1		89.09	40.44	7.92	35.92	15.72	0
Arginine	6	14	2	4		174.20	41.37	8.10	18.37	32.16	0
Aspartic acid	4	7	4	1		133.10	36.10	5.30	48.08	10.52	0
Cysteine	3	7	2	1	1	121.16	29.74	5.82	26.41	11.56	26.47
Cystine	6	12	4	2	2	240.30	29.99	5.03	26.63	11.66	26.69
Glutamic acid	5	9	4	1		147.13	40.82	6.17	43.50	9.52	0
Glycine	2	5	2	1		75.07	32.00	6.71	42.63	18.66	0
Histidine	6	9	2	3		155.16	46.45	5.85	20.62	27.08	0
iso-Leucine	6	13	2	1		131.17	54.94	9.99	24.39	10.68	0
Leucine	6	13	2	1		131.17	54.94	9.99	24.39	10.68	0
Lysine	6	14	2	2		146.19	49.30	9.65	21.89	19.16	0
Methionine	5	11	2	1	1	149.21	40.25	7.43	21.45	9.39	21.49
Phenylalanine	9	11	2	1		165.19	65.44	6.71	19.37	8.48	0
Proline	5	9	2	1		115.13	52.16	7.88	27.79	12.17	0
Serine	3	7	3	1		105.09	34.29	6.71	45.67	13.33	0
Threonine	4	9	3	1		119.12	40.33	7.62	40.29	11.76	0
Tyrosine	9	11	3	1		181.19	59.66	6.12	26.49	7.73	0
Valine	5	11	2	1		117.15	51.26	9.46	27.32	11.96	0
Ornithine	9	19	6	3		265.26	40.75	7.22	36.19	15.84	0

Table 5.3. Summary of published amino acid composition data for *Euphausia superba*. Ala=alanine, Arg=arginine, Asp=aspartic acid, Cys=cysteine, Cyt=cystine, Glu=glutamic acid, Gly=glycine, His=histidine, Hyd=hydroxypropline, Ile=iso-leucine, Leu=leucine, Lys=lysine, Met=methionine, Phe=phenylalanine, Pro=praline, Ser=serine, Thr=threonine, Try= tryptophan, Tyr=tyrosine, Val=valine, Tau=taurine, Orn=ornithine. [1] Freeze dried krill. [2] % total protein. [3] Krill paste. [4] Krill meal [5] % total nitrogenous material. [6] % Mol. [7] Dried muscle. [8] % total amino acids. [9] protein hydrolysate. [10] % amino N. [11] Free amino acids % of total. [a] Abdominal segment, [b] digestive gland, [c] remaining body. [d] Bransfield Straight, [e] West of the South Orkneys, [f] East of the South Orkneys, [g] South Georgia (Jan), [h] South Georgia (Feb). *may contain iso-leucine.

		Ala	Arg	Asp	Cys	Cyt	Glu	Gly	His	Hyd	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Try	Tyr	Val	Tau	Orn
Savage & Foulds (1987)	[1,2]	5.4						2.5		5.4	6.6	5.9	2.1	4.1			3.9			4.4			
Top Ocean Inc. (2005)	[1,2]	6.0	5.7	9.0			11.5	7.8	3.3		6.7	8.5	8.8	2.1	4.4	4.0	6.3	5.1		6.0	7.9		
	[3]	5.6	7.6	11.2			15.8	4.4	2.4		4.9	8.2	9.5	3.7	5.0	4.0	4.1	3.8		4.4	5.1		
Suyama et al. (1965)	[2]	5.5	6.2	12.2		1.5	14.6	4.7	2.3		5.1	7.8	8.6	3.0	6.5	4.2	5.0	4.7	1.5	4.1	5.9		
Krill Canada (2005)	[2]	5.8	6.7	11.0	1.2		13.8	4.8	2.5		5.0		8.2	4.0	5.2	4.0	4.3	4.7		4.5	5.3		
A-Bank (2005)	[2,4]	5.8	6.7	11.0	1.2		13.8	4.8	2.5		5.0	7.8	8.2	4.0	5.2	4.0	4.5	4.7		4.5	5.3	2.9	
Rehbein (1981)	[2,4]	5.5		10.4	1.2		13.0	5.2	1.8	5.4	5.0	7.6	6.4	2.6	4.7	3.9	3.8	4.2	1.4	3.5	5.2		
McWhinnie et al. (1981)	[3,5]		6–13	11–13			11–13					6–13	6–13		6–13		2.7						
Schmidt et al. (2004)	[6] a ♂	8.8	7.6	13.2			11.7	15.0	1.1		2.8	6.3	6.4	0.6	3.0	4.4	5.2	4.0		2.5	3.2	4.0	
	♀	9.2	10.0	13.0			11.1	11.8	1.1		3.1	6.5	6.8	0.8	3.0	6.6	5.2	4.2		2.7	3.4	1.6	
	b ♂	8.8	5.4	13.3			10.6	11.8	0.8		3.1	6.9	7.4	0.6	3.2	5.3	5.7	4.9		3.1	4.3	4.8	
	♀	12.3	5.7	12.6			9.6	8.5	1.6		4.0	7.1	7.4	0.4	3.4	5.2	5.8	5.1		2.8	4.8	3.6	
	c ♂	9.0	6.3	12.8			11.1	14.3	1.0		2.9	6.3	6.5	0.8	3.6	5.0	5.6	4.2		2.7	3.5	4.5	
	♀	10.9	6.4	13.0			10.7	10.0	1.5		3.5	6.6	7.0	0.4	3.7	5.8	5.8	4.8		2.7	4.0	3	
Zhu & Wang (1989)	[7,8] ♂	5.7	6.9	10.4		1.1	13.8	6.3	1.9		7.5	8.1	7.7	2.4	4.4	6.2	4.5	4.6		4.2	4.4		
	♀	6.1	6.8	10.3		1.3	13.7	5.8	2.0		7.2	8.3	7.8	2.7	4.2	5.5	4.6	4.9		4.2	4.6		
Aqua-In-Tech Inc. (2005)	[9]	6.2	6.4	11.4			15.6	5.6	4.4		4.4	8.2	9.3	2.3	4.6	3.7	4.5	4.8		4.2	4.8		
Burkholder et al. (1967)	[9,10]	6.9	7.5	10.4		1.6	15.7	8.6	2.4		4.5	8.7	9.2	3.5	6.1	5.6	4.6	4.9	1.9	5.2	6.1		
Srinivasagam et al. (1971)	[9]	6.0	8.0	11.0		4.0	12.0					15.0*	12.0		6.0		2.0	7.0	2.0	7.0	8.0		
		5.6	7.5	10.7		1.2	15.2	4.6	3.5		5.1	7.7	10.0	2.8	5.0	3.3	3.8	4.3		4.3	5.2		5.6
	[11]	10.2	8.1	0.9	1.8		1.0	14.0	1.9		3.1	2.6	8.4	0.5	0.9	8.3	2.1	3.8	2.0	3.1	4.1	15.9	7.5
Sidhu et al. (1970)	[2,9]	6.1	6.8	13.3	1.8	1.5	18.3	5.3	2.5		5.7	8.3	11.5	2.8	5.1	3.8	4.8	5.0	1.3	5.9	5.3	6.1	
Ferguson & Raymont	[9 d]	6.3	7.3	10.4	2.4	0.1	11.8	4.8	2.5		5.6	7.5	9.8	2.6	4.7	3.2	4.5	4.2		5.3	5.5	0.8	0.4
(1974)	[e]	5.8	7.7	10.7	0.9	1.2	11.9	4.7	2.8		5.3	7.9	10.5	2.8	5.0	3.4	4.7	4.4		4.8	5.5	0.7	
	[f]	6.5	6.9	11.0	0.9	0.9	12.7	4.5	2.6		5.7	8.1	10.0	2.2	4.8	3.1	4.8	4.6		4.5	5.4	0.9	0.6
	[g]	5.7	6.8	11.4	1.1	1.1	12.5	4.3	2.7		5.9	8.2	9.5	2.6	5.0	3.2	4.7	4.5		4.8	5.4		0.8
	[h]	6.8	6.9	10.5	0.7	0.7	13.3	4.5	2.6		6.1	8.0	9.1	2.2	4.9	3.0	5.3	5.1		4.4	5.6	0.2	0.8

Lipid

All lipids are carbon rich molecules, but there are significant differences in the C : N : P ratios between different classes of lipid. Fatty acids and glycerol (and hence triacylglycerols, waxes and sterols) contain no N or P, whereas phospholipids contain P and frequently N, contributing significantly to the total amount of P present in animals. Given these differences in the elemental chemistry of different lipids, the composition of the different classes of lipid in an organism can potentially significantly affect its overall stoichiometry.

There have been a plethora of papers investigating the lipid composition of *Euphausia superba* over the years, some of which include very detailed breakdowns of fatty acid compositions, recently reviewed in Falk-Petersen et al. (2000). Within the scope of this project, I will not attempt to summarise all available data, however, Table 5.4 gives an idea of the main components of lipid found in krill.

In terms of describing a stoichiometric composition of a generalised krill lipid, a simplification has been applied that assumes that krill lipid is 50% phospholipid and 50% triacylglycerol. Work previously undertaken by Clarke (unpub. data) has shown a negative relationship between the amount of total lipid and the phospholipid fraction of that lipid in individual krill (see Figure 5.1). Although there are stronger relationships when the data are separated by sexual maturity stage, the trend for all life history stages remains significant (ANOVA, $F_{1, 182} = 23.47$, $p < 0.001$). If the mean value for lipid content from Chapter 3 (6.42% fresh mass, all krill) is solved for this relationship, the phospholipid component of this lipid is estimated to be 46.9%. The next most numerous component in krill lipid is triacylglycerol (Bottino, 1975; Clarke, 1980), with Ju and Harvey (2004) showing triacylglycerols to represent an average of 45.5% of the total lipid in Antarctic

krill. It is therefore assumed for the 'generalised krill lipid', that 50% is phospholipid and 50% triacylglycerol.

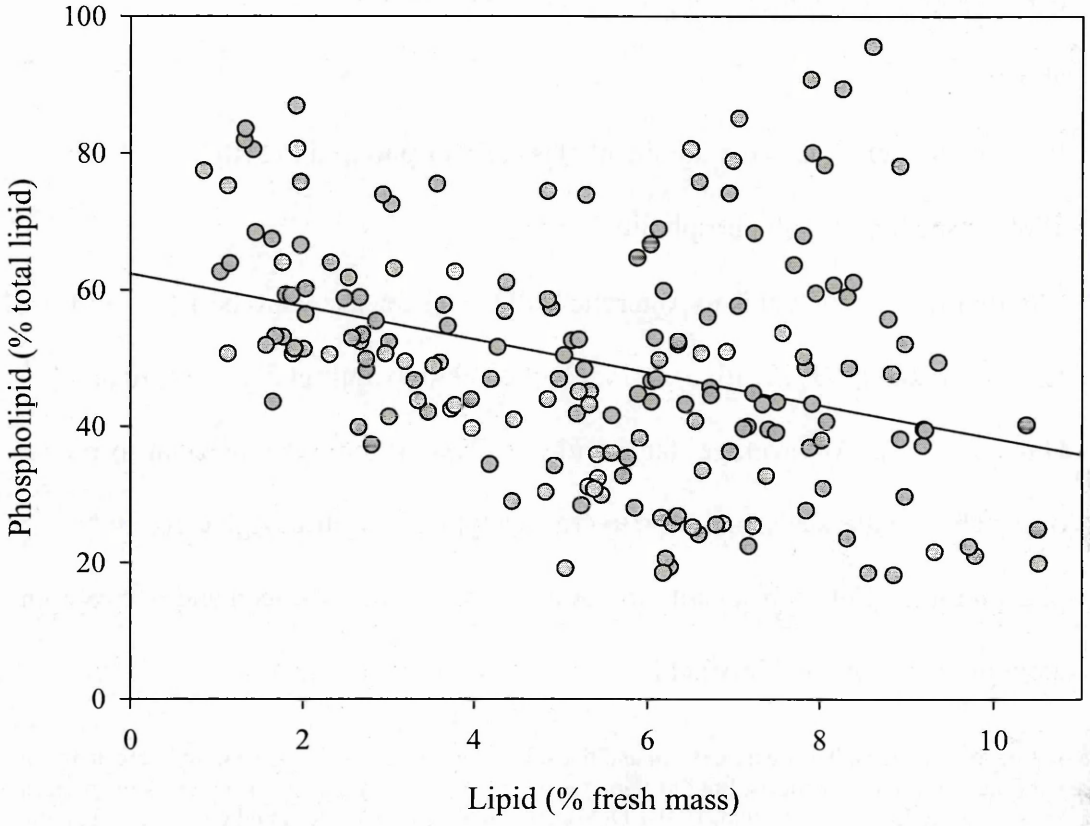

Figure 5.1. The relationship between the lipid content of *Euphausia superba* and its constituent proportion of phospholipid. R^2 = 11.4%. Data plotted from Clarke, unpublished data.

Constructing a formula for a molecule of 'generalised krill lipid' is not straightforward, as the molecular formula for complex lipids such as phospholipids and triacylglycerols depends on the fatty acids they contain. Krill have been shown to contain a wide range of saturated, monounsaturated, polyunsaturated and branched fatty acids ranging from C12 to C22 (Ju & Harvey, 2004). The composition of these fatty acids is highly variable with sexual maturity stage and food availability, with diet having the greater effect on composition (Cripps et al., 1999). This wide range of variation, strongly influenced by diet, makes it hard to select a fatty acid profile for a generalised krill lipid, based on a specific study from previously published data. In addition, there are several classes of phospholipid, with varying C : N : P ratios.

Therefore, there are several underlying assumptions made in order to construct this 'generalised krill lipid':

- That triacylglycerols are the main neutral lipid, and phospholipids the main complex polar lipid, each comprising 50% of the total lipid (by mass), as discussed above.
- Phosphatidyl choline is the dominant class of phospholipid in krill (~ 80% Clarke, 1980), used here for all phospholipid.
- The four major fatty acids in Antarctic krill have been shown to be 14:0, 16:0, 18:1 (ω-9) and 20:5 (ω-3) (Bottino, 1975; Clarke, 1980; Cripps et al., 1999; Ju & Harvey, 2004). An 'average' fatty acid was derived from the molecular formulas of the above fatty acids, and used to create a typical krill triacylglycerol and phospholipid. For each substitution of an R group, one hydrogen and one oxygen atom are lost from the fatty acid.

Table 5.4. Carbon, nitrogen, hydrogen, oxygen and phosphorus composition (molecular and percentage of total mass) of the major components of lipid in *Euphausia superba*. Triacylglycerol is given as the molecular formula without the addition of fatty acids (CH_2COOR-$CHCOOR'$-CH_2-$COOR''$) and phosphatidyl choline as $(CH_3)_3N$-CH_2-CH_2-PO_4-CH_2-$CHCOOR$-CH_2COOR'.

	Molecular formula					Molecular	% by mass				
	C	H	O	N	P	mass	C	H	O	N	P
Glycerol:	6	5	6			173.10	41.63	2.91	55.46	0	0
Phosphoglycerol-choline	10	18	8	1	1	311.23	38.59	5.83	41.13	4.50	9.95
Fatty acids:											
14:0 myristic acid	14	28	2			228.37	73.63	12.36	14.01	0	0
16:0 palmitic acid	16	32	2			256.42	74.94	12.58	12.48	0	0
18:1 (ω-9) oleic acid	18	34	2			282.46	76.54	12.14	11.33	0	0
20:5 (ω-3) eicosapentaenoic acid	20	30	2			302.45	79.42	10.00	10.58	0	0
'average' krill fatty acid	17	31	2			267.43	76.35	11.68	11.97	0	0
Generalised TAG	54	95	6			840.33	77.18	11.40	11.42	0	
Generalised PL	42	78	8	1	1	756.04	66.72	10.40	16.93	1.85	4.10
Generalised krill lipid							71.95	10.90	14.18	0.93	2.05

Nucleic acids

Nucleic acids comprise a sugar and phosphate backbone combined with any one of five nitrogenous bases (adenine, guanine, cytosine, thymine in DNA, uracil replacing thymine in RNA) in the ratio of one sugar-phosphate backbone complex to one nitrogenous base. The nitrogen content of the bases varies from 21.5% in thymine to 51.8% in adenine. The base composition of *Euphausia superba* has been investigated by Machida et al. (2004), and comprises 34.2% T (or U), 33.5% A, 18.4% C and 13.8% G. The composition for a 'standard krill DNA or RNA molecule' was calculated using the molecular masses of the bases in the above proportions and the sugar-phosphate backbone. However, it can be seen that the change in nucleotide composition has little effect in the overall stoichiometry of RNA and DNA (see Table 5.5).

Table 5.5. Carbon, nitrogen, hydrogen, oxygen and phosphorus composition (molecular and percentage of total mass) of the components of DNA and RNA. All bases given with one less hydrogen to allow for loss on bonding to backbone. Comparison of different base pair compositions on the molecular formula of DNA and RNA molecules. 'Krill DNA/RNA' assumes the base composition given in Machida et al. (2004).

	Molecular formula					Relative molar	% by mass				
	C	H	O	N	P	mass	C	H	O	N	P
DNA sugar-phosphate backbone	5	7	5		1	178.08	33.72	3.96	44.92	0	17.39
RNA sugar-phosphate backbone	5	7	6		1	194.08	30.94	3.64	49.46	0	15.96
Thymine	5	5	2	2		125.11	48.00	4.03	25.58	22.39	0
Adenine	5	4		5		134.12	44.78	3.01	0	52.22	0
Cytosine	4	4	1	3		110.09	43.64	3.66	14.53	38.17	0
Guanine	5	4	1	5		150.12	40.00	2.69	10.66	46.65	0
Uracil	4	3	2	2		111.08	43.25	2.72	28.81	25.22	0
One molecule of:											
DNA:											
5% C-G, 95% A-T						615.43	38.93	3.76	31.20	16.05	10.07
95% C-G, 5% A-T						616.32	37.12	3.61	31.15	18.07	10.05
'krill DNA'						613.80	38.42	3.73	31.32	16.45	10.09
RNA:											
5% C-G, 95% A-U						317.05	35.99	3.35	35.32	15.57	9.77
95% C-G, 5% A-U						323.81	35.24	3.42	34.59	17.19	9.57
'krill RNA'						318.76	35.70	3.58	35.17	15.84	9.72

Energetic nucleotides

Adenosine triphosphate (ATP) is a nucleotide that is present in cells to store and transport energy. Although not a significant contribution by mass to proximate composition, it is potentially a high source of phosphorus in animal tissue, because it contains the highest percentage of phosphorus (18% by mass, Table 5.6) of any major biochemical fraction, and as such should be included in these calculations. Because ATP occurs in equilibrium with adenosine diphosphate (ADP) in the presence of phosphorus, only ATP concentrations are considered here.

ATP was not determined analytically and no reference exists as to the proximate concentration of ATP in krill tissue (although daily ATP usage has been estimated for metabolic calculations). However, Båmstedt (1986) collected data for marine copepods, which ranged from 0.3% to 1.8% ATP (dry mass), with a mean of 0.7%. This mean value was adopted here for use as a proxy for krill ATP concentration, in the absence of more appropriate data.

Table 5.6. Stoichiometric composition (by mass) of the proximate biochemical fractions in *Euphausia superba*. Molecular masses used for calculation as follows: C = 12.0107, H = 1.00794 and N = 14.0067 (Lide, 2002). † less one hydrogen to account for loss on polymerisation. * not determined. ‡ Below analytical detection.

Component	Molecular formula	Molecular mass	Percentage by mass			
			C	H	N	P
Water	H_2O	18.02	—	11.19	—	—
Chitin (N-acetyl-glucosamine)	$C_8H_{15}N0_6$	220.20†	43.64	6.41	6.36	—
Mineral ash	From CHN data	—	0.24	0‡	0‡	*
Carbohydrate (glycogen)	$(C_6H_{10}O_5)_n$	162.14	44.45	6.22	—	—
Generalised krill protein	See text	—	45.32	7.55	13.91	—
Generalised krill lipid	See text	—	71.95	10.90	0.93	2.05
Generalised krill DNA	See text	613.80	38.42	3.73	16.45	10.09
Generalised krill RNA	See text	318.76	35.70	3.58	15.84	9.72
Adenosine triphosphate	$(C_{10}H_{16}N_5O_{13}P_3)$	507.18	23.68	3.18	13.81	18.32

Table 5.6 shows a summary of the stoichiometric composition (by mass) of each proximate biochemical fraction. In order to compare the experimentally derived elemental composition data with those derived by proximate composition analysis, the stoichiometric

composition for a whole krill had to be calculated. This was done by adjusting the elemental mass values for each biochemical fraction by the fresh mass it represented in a whole krill (data from Chapter 3) (as a percentage of proximate analysis totals, Table 5.1) and repeated for each sexual maturity stage. To compare with experimentally derived elemental composition data, expressed as % dry mass, the contribution of water fraction was subtracted from the total. Results are shown in Table 5.7.

Table 5.7. Comparison of stoichiometric data (% dry mass) for the composition of *Euphausia superba* derived from elemental content analysis and proximate biochemical analysis. *No value for carbohydrate, so mean value for all data (0.39%) substituted in calculation.

	Elemental analysis data				Stoichiometric approximation			
	C	H	N	P	C	H	N	P
All data	48.2	7.37	9.32	1.29	46.92	7.39	8.07	0.84
J	47.4	7.26	9.57	1.26	46.38	7.32	8.11	0.84
MS1	47.9	7.30	9.27	1.29	46.77	7.36	8.05	0.86
MS2	49.2	7.56	8.98	1.28	48.57	7.62	7.51	0.90
MS3	52.9	7.82	8.13	1.22	47.78	7.48	7.63	0.85
MS	*48.4*	*7.40*	*9.15*	*1.28*	*47.60*	*7.48*	*7.77*	*0.87*
MA1	51.2	7.71	8.50	—	49.88	7.80	7.10	0.95
MA2	43.1	6.80	10.88	1.17	41.32	6.61	9.31	0.52
MA	*45.8*	*7.11*	*10.08*	*1.17*	*43.73*	*6.94*	*8.67*	*0.64*
FS	48.5	7.37	9.20	1.30	47.66	7.49	7.75	0.89
FA3*	47.0	7.24	9.90	1.25	49.27	7.73	8.06	0.99
FA4	50.2	7.59	10.08	1.34	47.89	7.56	8.45	0.75
FA	*48.6*	*7.41*	*9.89*	*1.29*	*48.11*	*7.59*	*8.46*	*0.89*

These stoichiometric approximations fall within 5% of the measured elemental value for carbon (with the exception of MS3, within 10%) and hydrogen, but nitrogen and phosphorus are less well explained, lying outside 10% of the variation.

In atomic terms (normalised to P) the stoichiometric composition determined for *Euphausia superba* derived from elemental analysis was thus (oxygen excluded):

$$C_{96}H_{176}N_{16}P$$

and estimated from proximate biochemical analysis was:

$$C_{144}H_{271}N_{21}P$$

Expressed by mass the elemental composition measured directly was:

$$C_{4.01}H_{7.31}N_{0.67}P_{0.04}$$

and from proximate analysis was:

$$C_{3.91}H_{7.33}N_{0.58}P_{0.03}$$

for an average krill caught near South Georgia in January.

Although the results shown in Table 5.7 initially are broadly consistent, it can be seen than the values derived by elemental analysis are higher (with the exception of hydrogen). This could indicate that the stoichiometric approximations for proximate composition applied were not sufficiently accurate. In particular the derivation from proximate composition appears to underestimate phosphorus and nitrogen.

The largest contribution to the total phosphorus content is the lipid fraction (of which only the phospholipid component contains any P or N). This could indicate that the phospholipid contribution to the total lipid is too low. Indeed, Figure 5.1 shows that phospholipid contents can rise to over 80% of total lipid in some krill. If the ratios of phospholipids to triacylglycerols are adjusted, the results presented in Table 5.8 are obtained (data for all krill used only).

Table 5.8. The effect on the stoichiometry of *Euphausia superba* of changing the ratios of phospholipid (PL) to triacylglycerol (TAG). All krill = data from elemental analysis.

Ratio	Stoichiometric approximation			
PL:TAG	C	H	N	P
50:50	46.92	7.39	8.07	0.84
60:40	46.64	7.37	8.12	0.95
70:30	46.35	7.34	8.17	1.06
80:20	46.07	7.31	8.22	1.17
90:10	45.79	7.29	8.27	1.28
100:0	45.51	7.26	8.32	1.39
All krill	48.2	7.37	9.32	1.29

This has the effect of increasing the phosphorus and nitrogen fractions, although the carbon content is reduced. From these data, the ratio of phospholipid to triacylglycerol

would have to be 90:10% total lipid to explain the N and P values determined by elemental analysis, which we can see from Figure 5.1 might be true for some individuals, but is unlikely to represent the majority.

The second largest contributor to total phosphorus, despite its potentially small mass fraction, is ATP. It is very likely that there will be errors associated with the estimation of the total ATP content, as a mean value was used from a synthesis of copepod data. Additionally, the same ATP fraction was assigned to all sexual maturity stages. By varying the assumed mass of ATP in krill, it is clear that an increase in the amount of ATP increases all stoichiometric elemental fractions (see Table 5.9). An ATP concentration of 3% (dry mass) brings the phosphorous, hydrogen and carbon concentrations almost in line with those obtained by direct elemental analysis, although this is outside the range observed for copepods in Båmstedt (1986) and higher than the maximum observed value for insects of 2% (DeZwann & van den Thillart, 1985; in Sterner & Elser, 2002).

Table 5.9. The effect on the stoichiometry of *Euphausia superba* of changing concentration of the nucleotide adenosine triphosphate (ATP) (expressed as dry mass). All krill = data from elemental analysis.

ATP	Stoichiometric approximation			
(% DM)	C	H	N	P
0.3	46.82	7.38	8.01	0.77
0.7	46.92	7.39	8.07	0.84
1.8	47.18	7.43	8.22	1.04
2.5	47.34	7.45	8.32	1.17
3.0	47.46	7.47	8.38	1.26
All krill	48.2	7.37	9.32	1.29

Whilst a combination of phospholipid and ATP may explain the error in P, the underestimation of nitrogen is almost certainly caused by an error in the protein term, as 88% of the nitrogen fraction of krill explained by this proxy method is attributable to protein. If, instead of the generalised protein derived by this study, the protein stoichiometry derived from the amino acid compositions of bacteria, algae and aquatic animals, as devised by Gnaiger and Bitterlich (1984) (52.9% C, 7.0% H and 17.3% N) is

used, nitrogen (and carbon) increases significantly. This figure is, however, higher than those obtained by direct elemental analysis, suggesting that this overall value actually overestimates krill protein nitrogen. Applying the commonly used figure of 16% nitrogen in protein from Lusk (1928) as cited in Gnaiger and Bitterlich, produces a figure for nitrogen more comparable to that directly determined.

Table 5.10. The effect on the stoichiometry of *Euphausia superba* of changing the nitrogen composition of protein.

	Stoichiometric approximation			
Protein type (N%)	C	H	N	P
Generalised krill protein (13.9)	46.92	7.39	8.07	0.84
Gnaiger and Bitterlich protein (17.3)	50.71	7.11	9.76	0.84
Traditional estimate (Lusk) (16.0)	—	—	9.11	—

The low estimate of nitrogen in krill protein, indicates that the choice of amino acid composition was potentially not suitable (although levels of amino acids high in nitrogen – alanine, cystine and lysine are not significantly different from those in other studies), or possibly that the free amino acid pool needs to be included into the estimate of protein.

In attempting to resolve the slight mismatch between observed and calculated elemental compositions, we also need to consider the effect of experimental error. Most importantly, the two sets of data being compared do not originate from the same animals. The comparison would be greatly improved if both elemental and the full suite of proximate analysis could have been undertaken on individual krill. Also, both methods of analysis are subject to experimental error, and this error is propagated through the calculations. An idea of the cumulative variance in the proximate biochemical data set can be gained from the data in Table 5.1, where the variance in the total figure exceeds that of any single component.

The influence of sampling on different individuals is highlighted in the results for the different life history stages. For example, the increase in carbon above the average in male MA1 krill measured by the CHN analysis is reflected in the elemental composition

estimated from stoichiometric approximation of the biochemical data, and is caused by the observed high lipid concentrations in these adult krill. Furthermore, when patterns in the elemental composition are not reflected in the biochemical data it is typically a result of low replicate numbers magnifying the effect of variability. For example, male sub-adult krill, MS3, show high values of carbon which are not reflected in the data from the biochemical composition; this is because only one animal from this stage was analysed using the elemental analyser, and similarly low numbers of replicate krill were used in the biochemical analyses.

Applying the stoichiometric concept

Thus moving from a measured proximate composition data to an estimated elemental composition is relatively straightforward, if not entirely accurate; however reversing this step is less so. Nevertheless, the ability to move from a measured elemental composition data to an estimated proximate composition has many useful applications, not least in the field of energy budget modelling. Obtaining elemental composition data is quicker, and requires less material, than carrying out at suite of proximate composition analyses and therefore potentially allows for the analysis of greater numbers of samples and increased replication. Changes in the proximate composition of organisms are important though, as even when carbon, nitrogen and hydrogen concentrations remain relatively constant in an organism, their allocation between the various biochemical compounds varies with the sex, maturity and physiological state of the individual. Understanding this partitioning of elements between biochemical compounds and the degree of flux between them in response to life history and environmental changes will help to answer fundamental ecological questions and better understand the functioning of marine ecosystems.

This approach has been used by several authors, applying the stoichiometric equations of Gnaiger and Bitterlich (1984) to convert elemental composition to proximate

biochemical data (Riccardi & Mangoni (1999) on freshwater zooplankton, Zamer et al. (1989) on sea anemones, Clarke et al. (1992) on gelatinous zooplankton and Galley et al. (2005) on an echinoid). Gnaiger and Bitterlich (1984) applied stoichiometric coefficients to represent the composition of the soft tissues of fish and molluscs in terms of their three major biochemical fractions; protein, lipid and carbohydrate. However, in order for accurate results to be obtained when applied to other species by other authors, species specific adjustments have had to be made to these equations. The adjustments for *Euphausia superba* are discussed here.

Firstly, the equations of Gnaiger and Bitterlich (1984) were applied to data from this study without modification. An executable routine was used within MINITAB to apply these equations, included here as Appendix D. A brief summary of the algorithm is as follows:

- Convert dry mass to ash-free dry mass (as estimate of total organic matter).
- Correct ash-free dry mass for residual water (default value 6%).
- Correct total carbon for inorganic carbon retained as carbonate in ash (default value 0.1% in ash).
- Correct total nitrogen for fraction of non-protein nitrogen. The latter is principally nucleic acids (DNA plus RNA, default value 5% of total N), but in crustaceans this also includes chitin. This correction ignores N in phospholipid.

These corrections produce estimates of protein-nitrogen, organic carbon and organic hydrogen, all as a percentage of dry organic matter.

- Estimate total protein from protein-nitrogen.
- Estimate protein-carbon from this protein

- Subtract protein-carbon from total organic carbon, and express as a percentage of protein-free organic matter. The latter is assumed to be comprised solely of lipid and carbohydrate.
- Partition remaining organic carbon between lipid (78% C) and carbohydrate (44% C) by linear interpolation.

The routine then checks calculations by summing estimated proximate composition, and estimating residual water not explained by protein, lipid and carbohydrate hydrogen.

Table 5.11. Comparison of mass fractions of the elemental CHN composition of standard carbohydrate, lipid and protein reference compounds from Gnaiger and Bitterlich (1984) and the generalised krill composition derived earlier in this chapter.

	Gnaiger and Bitterlich			Generalised krill composition		
	C	H	N	C	H	N
Carbohydrate	0.444	0.062	0.000	0.444	0.062	0.000
Lipid	0.776	0.114	0.000	0.720	0.109	0.093
Protein	0.529	0.070	0.173	0.453	0.076	0.139

The executable routine was first run using the assumption in Gnaiger and Bitterlich (1984) that all nitrogen is attributable to the nitrogen in protein and the residual water content of the dry tissue is 6%. When compared with the experimentally derived proximate composition data it was clear that this method significantly overestimated the protein and carbohydrate fractions (see Figure 5.3). This overestimation of the protein fraction occurs because not all krill nitrogen is in protein; significant amounts are found in other major biochemical components (principally chitin, nucleic acids and lipid). The overestimate of carbohydrate can be attributed to the large amounts of chitin present in krill which, without modification, is assigned to carbohydrate using this method.

The routine was then re-run, using the previously calculated percentage of non-protein nitrogen (13.7% of total N) and the concentration of nitrogen in this non-protein nitrogen (11.3%). The amount of residual water in dry krill tissue was not known, so the default value described by Gnaiger and Bitterlich (1984), as above, was used in the absence of data for krill. However, it should be noted that the non- protein nitrogen is not

constant and will exhibit variation between individuals related to sex, maturity, condition and environmental conditions (Riccardi & Mangoni, 1999 and Chapter 3). A third run was therefore undertaken, applying the sexual maturity stage specific percentage non-protein nitrogen for each particular life history classification. It is a reasonable assumption that the composition of non-protein nitrogen is unchanged, even though the amount of that non-protein nitrogen is sex/stage specific (see Table 5.12).

Estimations of proximate compositions are only as accurate and precise as the input data allow and how well the calculated generalised composition for the protein, carbohydrate and lipid represent those in the real animal. Any errors in the CHN data are magnified by the calculation procedure, especially those relating to hydrogen, which are inherently subject to a greater degree of variability related to analytical procedure and which are highly sensitive to residual water. In an attempt to reduce this variability, only the mean CHN values for each life-history classification were analysed using the proxy method.

Table 5.12. Comparison between the proximate composition of krill, *Euphausia superba*, derived by analytical means, the stoichiometric method of Gnaiger and Bitterlich (1984) and the stoichiometric method modified for krill (using overall mean non protein nitrogen percentage applied to all data and sexual maturity stage specific % non-protein N). NPN = percentage non-protein nitrogen. All data expressed as % dry mass.

	Proximate analysis data			(Gnaiger & Bitterlich, 1984)			Modified			Individually modified			
	Protein	Lipid	Carbohydrate	Protein	Lipid	Carbohydrate	Protein	Lipid	Carbohydrate	Protein	Lipid	Carbohydrate	NPN
All	46.7	25.1	1.54	53.87	20.94	7.71	46.49	22.83	1.93	46.49	22.83	1.93	13.70
J	47.3	24.6	1.48	55.32	19.43	6.83	47.74	21.37	0.88	48.75	21.11	1.68	11.87
MS1	44.4	23.8	1.61	53.58	19.39	10.10	46.24	21.27	4.34	46.09	21.31	4.22	13.99
MS2	44.0	30.7	0.75	51.91	23.43	7.95	44.80	25.25	2.38	43.49	25.59	1.35	16.22
MS3	41.4	26.5	1.08	46.99	34.93	2.05	40.56	36.58	-3.00	37.96	37.25	-5.04	19.22
MS	*44.1*	*26.9*	*1.33*	52.89	20.92	9.37	45.64	22.78	3.69	44.78	23.00	3.01	15.33
MA1	44.4	36.8	1.38	49.13	30.65	3.15	42.40	32.37	-2.13	41.08	32.71	-3.17	16.39
MA2	55.3	11.5	1.80	62.89	7.63	8.72	54.27	9.84	1.96	56.38	9.30	3.61	10.35
MA	*51.3*	*17.6*	*1.63*	58.27	15.72	6.18	50.28	17.77	-0.08	51.16	17.54	0.60	12.20
FS	46.1	28.6	1.51	53.18	22.65	6.22	45.89	24.52	0.51	45.48	24.62	0.18	14.48
FA3*	44.3	27.6	1.92	57.23	13.92	13.29	49.39	15.93	7.15	48.85	16.07	6.72	14.64
FA4	48.0	23.7	1.54	58.27	21.76	5.56	50.28	23.81	-0.70	51.09	23.60	-0.06	12.31
FA	*47.5*	*24.3*	*1.54*	57.17	17.44	10.82	49.34	19.45	4.67	49.97	19.29	5.17	12.59

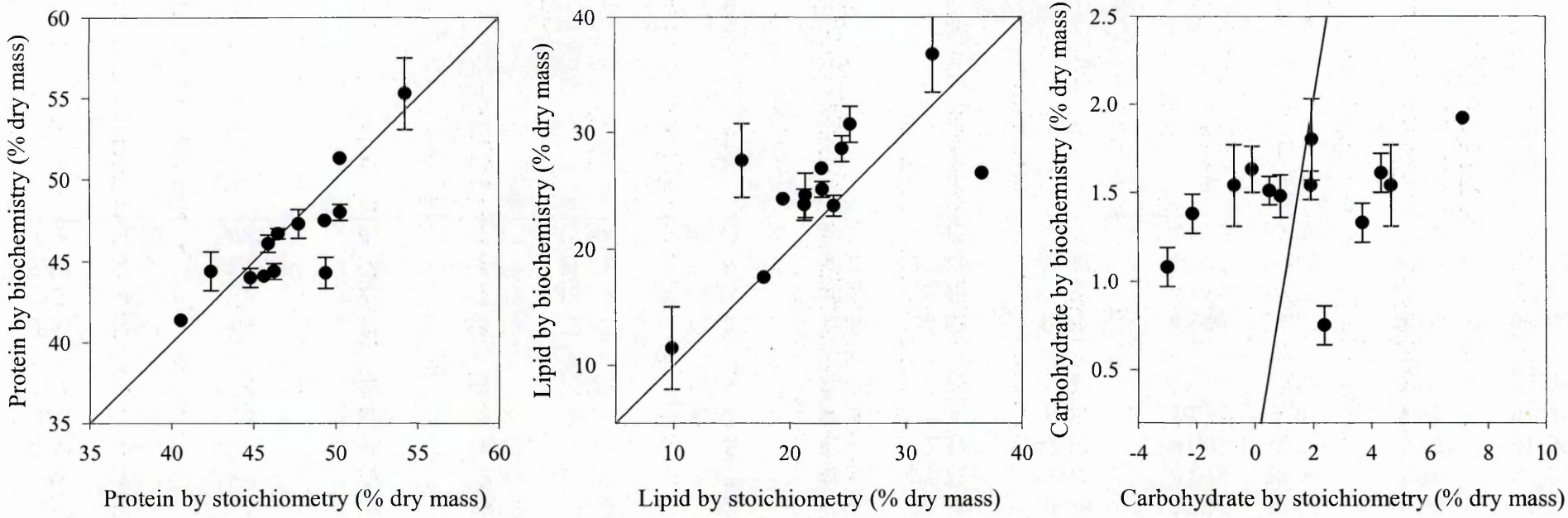

Figure 5.2. Comparison of biochemical and stoichiometric determination of proximate biochemical composition. Error bars are one standard error (only on y axis). Solid line represents a perfect match, proximate = stoichiometric. Each point represents a different sexual maturity stage.

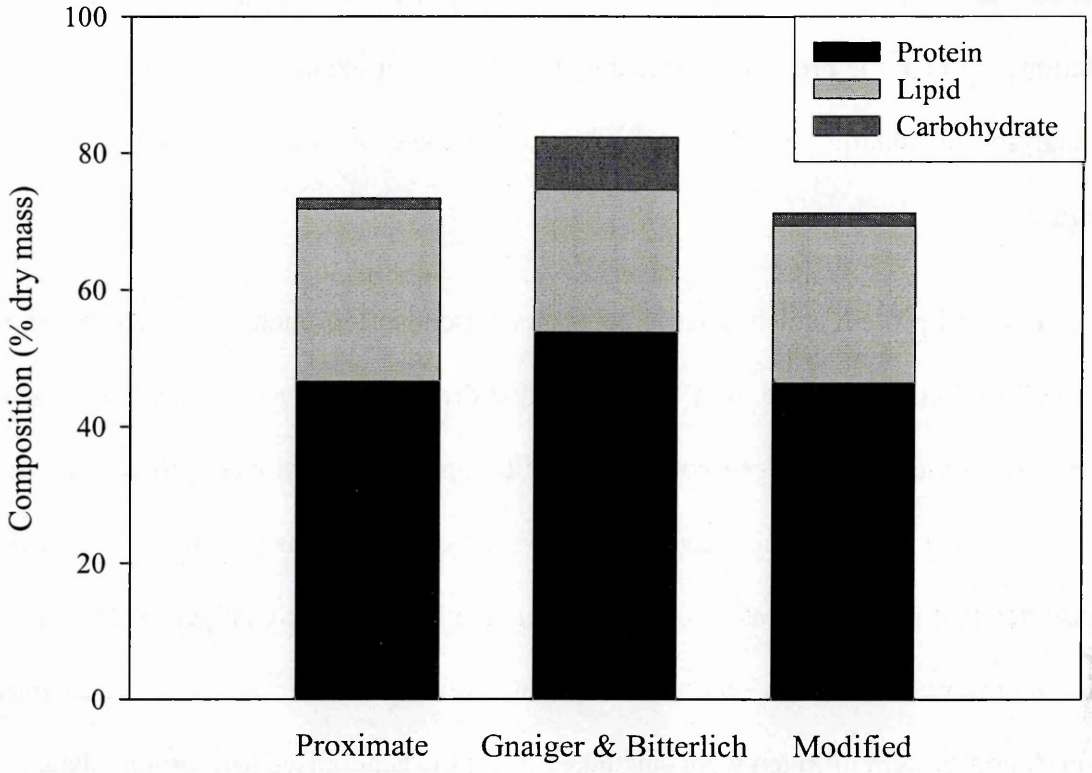

Figure 5.3. Proximate biochemical composition of an average krill, *Euphausia superba*, as determined by analytical techniques, the stoichiometric method of Gnaiger and Bitterlich and the modified Gnaiger and Bitterlich method using stoichiometric approximations specific to krill.

The principle problem with applying Gnaiger and Bitterlich's stoichiometric coefficients in an unmodified form to zooplankton, is the overestimation of carbohydrate; this is because the contribution of non- protein nitrogen from chitin (principle carbohydrate in zooplankton), is ignored. One solution to this would be to substitute the stoichiometric constants for chitin in place of those used for glycogen in the carbohydrate sections of the equations. However, this would then influence the estimation of the non-chitin carbohydrate, which although small in krill, is biologically important. Instead, by including chitin in the non-protein nitrogen fraction, it is excluded from the carbohydrate calculations, and the figures for mean values of carbohydrate are reasonably well explained. For the sexual maturity stage categories, however, the carbohydrate term contains errors. The reason that the carbohydrate data is not so accurately forecasted using this method (see Figure 5.2) is that carbohydrate is only a small fraction of the total mass

of krill, so errors are likely to be magnified. In addition the carbohydrate fraction is calculated last and so it accumulates all the stochastic errors from the preceding calculations. Where the precision of the data is reduced, for example when the number of krill analysed is small, then the carbohydrate estimate is imprecise and somewhat inaccurate.

Lipid and protein stoichiometric constants were also left unchanged. However, in the case of protein, we can see from Table 5.10 that the values for generalised krill protein underestimated the total nitrogen content, and although the 'traditional' protein nitrogen value was a closer estimate, the complete formula for krill protein is unknown. Figure 5.2 demonstrates that protein is reasonably well estimated by using the Gnaiger and Bitterlich protein approximation. However, the constants total non-protein nitrogen and mass fraction of non-protein nitrogen were calculated using the generalised krill protein data.

The estimate of residual water, 6%, as given in the original paper, seems to be appropriate for use on krill, as when this component is recalculated within the executable routine as an error checking mechanism, the mean value is given as 6.21%. This residual water component is very sensitive to errors in the experimental procedure, and care was taken during elemental analysis to follow the recommendations of Gnaiger and Bitterlich. In particular the material for analysis was re-dried after each stage (weighing, homogenising etc) and steps were taken to prevent samples gaining water on the carousel of the CHN analyser. It is encouraging to see that the uptake of residual water was minimised by these methods.

This method of determining proximate composition from elemental data is clearly an improvement of the traditional crude methods of estimating total protein from nitrogen. Using the value for generalised krill protein given in Table 5.6, gives a conversion ratio of 7.19 x N = protein. However, when applied to the N data from CHN analysis and

compared to the value of protein determined by biochemical analysis, it is clear that this figure gives a significant overestimation (67% protein compared with the observed value of 46.7%). Traditionally, the conversion factor of 6.25 has been used to estimate protein from N (references in Gnaiger and Bitterlich), which reduces the overestimate to 58.25%. Gnaiger and Bitterlich (1984) discuss how this figure underestimates the amount of protein nitrogen seen in typical amino acid compositions of bacteria, algae and aquatic animals, and propose a new nitrogen-protein conversion factor of 5.8, which they argue can be applied to aquatic organisms with an accuracy of 3%. This factor gives the closest estimate to our observed value; 54.06%. The stoichiometric method, as presented here, allows this value to be further modified by the total of non-protein nitrogen, where any non-protein nitrogen decreases this conversion factor.

Application of this method to JR82 elemental composition data.

In order to further test the application of this method, on krill that were sampled in a different location and year from those for which the method above was modified, elemental composition data from JR82 was analysed using the above method. The constants were the same as applied to JR70 data, with the mean value for percentage non-protein nitrogen (13.7%) applied to all sexual maturity stages. Table 5.13 summarises the results. Residual water was again seen to be a reasonable assumption, with a mean for this data set of 6.15%.

This proximate composition data reflects the differences between sampling years observed in the elemental composition analysis, as discussed in Chapter 3. The proximate composition of these krill appears reasonably well explained, using this proxy method, with the exception of the carbohydrate fractions which show a high degree of variability. However, differences between sexual maturity stages that would be predicted from our knowledge of the life history of krill have been detected. For example, there is an appreciable increase in lipid content in the mature adult female krill to a maximum at the

gravid stage FA4, which is then reduced in the post-spawn stage FA5. Similarly, differences between the sexual maturity stages shown through the analysis of the JR70 krill is confirmed here, for example the MA2 mature adult male krill have a very reduced total lipid content, (see Figure 3.10 and accompanying discussions).

Table 5.13. Estimation of the proximate composition of *Euphausia superba* collected during JR82 from elemental composition data, using the modified stoichiometric method of Gnaiger and Bitterlich (1984). S.E. = standard error, *n* = total number of krill analysed.

	Protein		Lipid		Carbohydrate		
	Mean	S.E.	Mean	S.E.	Mean	S.E.	*n*
All	50.14	0.32	15.25	0.81	2.21	0.54	
J	49.71	0.48	17.18	1.21	0.27	0.83	47
MS1	53.40	—	10.48	—	1.77	—	1
MS2	47.91	—	18.15	—	5.17	—	1
MA1	49.53	0.37	14.70	1.27	3.28	1.68	2
MA2	55.51	0.97	2.79	0.15	6.10	0.83	2
FS	41.62	—	30.79	—	0.54	—	1
FA1	52.07	0.55	10.83	1.61	4.22	1.23	25
FA2	51.59	0.27	10.51	2.87	5.79	2.18	3
FA3	50.75	2.52	12.89	3.51	3.67	1.04	3
FA4	49.22	0.47	20.25	1.73	2.99	1.30	12
FA5	46.98	0.66	14.75	2.20	2.65	1.22	8

DISCUSSION

Although this method shows clear promise for elucidating the patterns in proximate composition in individual krill, there are significant limitations. This technique is inherently sensitive to experimental errors in the elemental composition, total mineral ash and dry mass determinations. Any errors in these data are amplified during the calculations involved in the stoichiometric proxy method, and the smallest biochemical fraction, carbohydrate, is the most sensitive to these accumulated errors. Unfortunately, the data from this study were not suitable to compare direct proximate and elemental composition on the scale of an individual krill, as there was not sufficient material for accurate determination using the methods used here.

Further refining of this method to improve the accuracy of the predictions is not possible without a data set that includes elemental and proximate composition data from the same individual animals, as without this data it is not possible to have confidence in the accuracy or results of the manipulations. This should be an objective for further work, however, as the preliminary results from this study demonstrate clearly the potential for this technique.

The results from the JR82 data set are very encouraging: patterns of varying proximate composition with life history stage are clearly identified, and the data are within the ranges observed from direct chemical composition studies (see Chapter 3). It would seem likely that this technique could be applied to krill sampled from anywhere in the Antarctic during any season using the methods as described here without modification to give a broad idea of the proximate biochemical composition. However, if looking for individual scale changes, either over time or with experimental manipulation or environmental variability, further modification would be required.

The use of this technique should be encouraged, especially when considering ecosystem scale questions of energy transfer and food web dynamics. Elemental analysis should be undertaken to the standards described by Gnaiger and Bitterlich to ensure that experimental errors are minimised and the publication of hydrogen content data is encouraged to allow the wider application of this technique and the benefits of its application to be realised for studies of zooplankton ecology.

For future development of this approach, the Gnaiger and Bitterlich protocol would be improved by the inclusion of phosphorus, and the mechanisms for this should be explored.

Chapter 6 An energetics model for krill growth and reproduction.

INTRODUCTION

As previously discussed in Chapter 1, the Antarctic krill, *Euphausia superba* is of fundamental importance in the Antarctic ecosystem and we therefore need to be able to understand and predict its responses to environmental variability and change. Knowledge of the variability of the krill population helps us understand fluctuations in the food web, both in the higher predators (Murphy & Reid, 2001) and in interactions with lower trophic levels (Marchant & Murphy, 1994). Fluctuations in krill abundance can affect the foraging and breeding performance of predators and are linked to changes in the composition and dynamics of the plankton. For example, Croxall et al. (1999) observed that a four-fold reduction in krill biomass at South Georgia resulted in macaroni penguins (*Eudyptes chrysolophus*) switching diet from krill to amphipods and a 90% reduction in the breeding success of both black-browed albatross (*Diomedea melanophris*) and gentoo penguins (*Pygoscelis papua*). Atkinson et al. (1999) observed that in areas of high krill abundance around South Georgia, copepod numbers are unusually low, resulting either from competitive grazing or krill preying directly on copepods, or both. With robust population models we are better able to predict krill population growth and provide advice to CCAMLR (the Commission for the Conservation of Antarctic Marine Living Resources) to enable the setting of realistic catch limits for the krill fishery. The extrapolation from individual to population scale models is important to our understanding of ecosystem dynamics, but in order to construct accurate population and ecosystem scale models, we first need realistic and reliable models of individual growth; this in turn requires energetic models relating food intake to somatic growth and reproductive development.

Despite a large volume of work on the species over many years, there is much of the biology of Antarctic krill which remains unknown. Arguably, the most important

aspect for understanding the responses of krill to its environment, namely a detailed understanding of its energetics, is the area that still has the most questions to be answered. Knowledge of an animal's energy budget is essential for understanding its role and function in the ecosystem, and without adequately functioning energy budgets, we are hampered in our efforts to construct effective ecosystem models, an essential tool in modern ecological studies.

Several previous studies have made significant progress towards the goal of a predictive energy budget for larval (Frazer et al., 2002; Meyer et al., 2002; Meyer et al., 2003) and post-larval krill (Clarke & Morris, 1983; Miller & Hampton, 1989; Quetin et al., 1994; Atkinson et al., 2002; Meyer et al., 2003). These approaches, whilst estimating a general energetic demand for krill survival, do so in a static manner with average values calculated over a given period of time, typically for a season (most often the summer), and do not take into account changes in physiology reflecting environmental variation. A more detailed approach is to create a dynamic energy budget, which allows the various components of the energy budget to be tracked and modified over a time period of several years, and to predict the consequences of temporal environmental fluctuation on the survival and success of adult krill.

The development of an accurate energy budget for krill has been limited by the fundamental problem that most physiological studies on krill are undertaken in the laboratory, as it is often hard to obtain precise and accurate physiological measurements in the field. Problems associated with laboratory studies include the fact that experiments are inherently confounded by having to keep krill in limited volume containers, which, for a free living pelagic organism, may potentially cause significant physiological stress, or a modification in behaviour, or can lead to poor estimates of physiological rates through experimental artefacts (Quetin et al., 1994). Similarly, krill tend to live in swarms in the wild which may, or may not, confer an energetic advantage (Alonzo & Mangel, 2002; Ritz,

2002). Although attempts have been made to induce swarming in metabolic studies of krill (Swadling et al., 2005), these have not been successful, so it is realistic to assume that any energy budget developed for krill represents an individual animal in a non-swarming environment. Additionally, we cannot quantify the effect that the stress of catching the animal has on physiological experiments, although many studies attempt to control for this by holding the animals in controlled conditions for periods of time prior to experimentation (e.g. Ikeda et al., 1983); however, this in turn may induce a change in physiology or behaviour associated with captivity. Both Kawaguchi et al. (2006) and Tarling et al. (2006) showed a reduction in measured growth rate from the second day of incubation during instantaneous growth rate (IGR) experiments. McWhinnie and Marciniak (1964) found that the oxygen consumption of krill decreased with increased time in the laboratory post-capture, however this could be related to altered feeding conditions in captivity, recovery from shock, a factor of the moult cycle or acclimation by the krill to captive conditions. Additionally, there is the problem of the varying timescales over which physiological measurements are made, and hence energy budgets calculated, from hours (metabolic rate) to days or months (growth rates and spawning) (Atkinson et al., 2006); extrapolating results to enable a comparison between them is problematic. However, when comparing energetically derived growth rates with results from IGR measurements (see Chapter 4), this becomes less of a problem when compared with the monthly, seasonal or annual growth rate estimates previously derived. A further important limitation concerns important components of the energy budget (such as the metabolic cost of swimming) that are yet to be accurately quantified.

To date, previous energy budget studies have all been based around the traditional formulation of the energy budget following Ricker (1968):

Equation 6.1

$$C = P + R + U + F$$

where: C = total food or energy intake; P = production (energy required for somatic and reproductive growth); R = respiration; U = excretion; and F = faeces (Clarke, 1987b). This approach has its origins in work done for the International Biological Programme (IBP) predominantly on domesticated animals (Blaxter, 1967), and is based pragmatically on measurements that can easily be made experimentally, rather than the underlying physiology driving these responses.

There are several limitations to this form of the energy budget: in the model proposed in Equation 6.1; key physiological process which cost the animal significant amounts of energy are absent, or not identified explicitly. It also lacks explicit recognition of physiological processes absent in terrestrial animals but important in pelagic organisms. In order for the metabolic component of an energy budget to be correctly assessed, it is fundamental to understand the contribution of each of the component costs of metabolism: basal metabolism, swimming activity, costs of feeding and growth. This has to date, presented the greatest problem in formulating an accurate energy budget for krill.

It is arguably more meaningful to consider the production of new somatic and reproductive (gonad) tissue separately, as rates of synthesis are different for each type of tissue and are limited by different constraints. The costs to the animal of synthesising each type of tissue will likely be different as they have a differing biochemical composition. Unfortunately we do not have good data on what these differences are. Pelagic invertebrates like krill have to swim continuously (and hence expend energy) in order to maintain their position in the water column, a cost over and above the strict definition of basal metabolism, and which it is extremely difficult to quantify. Feeding also has a metabolic cost to the animal, which must additionally be accounted for. It is therefore more ecologically meaningful to consider metabolic rate (expressed as O_2 consumption) as portioned into several independent, but interlinked components: the respiratory costs of feeding, swimming, production of somatic and production of reproductive tissue on top of

the traditional idea of basal metabolism. This approach, proposed by Clarke, has been discussed in detail (Clarke, 1987b; Quetin et al., 1994; Clarke, in prep.) but not yet applied to a functioning energy budget for any organism.

Quetin et al. (1994) detailed an improvement to the traditional formulation of the energy budget which takes into count these additional costs, better representing the true physiology of the animal:

Equation 6.2

$$C = (P_s + P_g) + (R_b + R_a + R_f + R_s + R_g) + F + U$$

where P_s represents somatic growth (including the exoskeleton), P_g the production of gametes and growth of reproductive tissue, and the respiration (or metabolism) term is divided into separate components for basal or maintenance costs (R_b), the cost of activity (R_a), the costs associated with feeding (R_f) and the respiratory costs associated with the production of new somatic (R_s) and reproductive (R_g) tissue. Quetin et al. (1994) do not split the costs of activity associated with locomotion and feeding, simply having an R_a term; however, in the case of krill it is perhaps more appropriate to split these costs, as the animal has to continually swim, but may not continuously be feeding.

Each component of this formulation of the energy budget will vary with the life history stage of the animal, and with external environmental variability (temperature and food availability). Although Quetin et al. (1994) presented this approach to the energy budget formulation and comprehensively reviewed the literature available on krill physiology applicable for the formulation of such an energy budget, they did not attempt any numerical estimations. Applying the development of this novel approach to the energy budget formulation to krill using dynamic energy budget modelling techniques will allow us to develop detailed predictive models of krill energetics which can be used to examine an organism's response to environmental variability in detail.

Modelling is a useful tool in energetic studies, because it allows the integration of several dynamic variables to estimate the flow of energy or mass through an organism (Brown & Rothery, 1993). By developing a robust and physiologically realistic model of the energy budget for krill, it becomes possible to investigate the various mechanisms for survival under a range of environmental conditions and assess potential effect on the success of the population and, ultimately ecosystem dynamics. In order to develop realistic and accurate population scale models for krill, models must be based on accurate, individually based, energy budget models, to minimise the effect of the magnification of errors as the models are expanded to population, and ecosystem scales.

To date there exist only a few modelling studies focusing on adult Antarctic krill, (Astheimer et al., 1985; Hofmann & Lascara, 2000; Alonzo & Mangel, 2001; Fach et al., 2002; Atkinson et al., 2006; Candy & Kawaguchi, 2006). The first attempt to model krill growth was undertaken by Astheimer et al. (1985) and Astheimer (1986), who used the population growth curves of Mackintosh (1972a) to investigate the effects of variation in food supply and length of the growing season. This phenomenological model of individual krill growth, where food conditions were used to modify the optimal growth curve, did not explicitly include metabolic processes or any calculation of net production. Models based on von Bertalanffy growth curves were developed by Rosenberg et al. (1986) and Siegel (1987), and Murphy and Reid (2001) modelled krill population dynamics to generate length frequency distributions observed in predator diets throughout the summer using a seasonal von Bertalanffy growth curve modified by mortality and predator availability and prey selection.

Recent models have been based on experimentally derived growth rate data using the IGR method (described in Chapter 4), to model krill growth under various environmental conditions using temperature-dependant IMPs (Candy & Kawaguchi, 2006; Kawaguchi et al., 2006) and experimentally derived growth rates (Atkinson et al., 2006).

The model of Kawaguchi et al. (2006), however, did not allow for the separation of the effects of food and temperature on the seasonal trends in IGR and IMP. Candy and Kawaguchi (2006) do not model female krill, because of the physiological complexity of dividing incoming resources between reproductive and somatic growth (Kawaguchi et al., 2006). Atkinson et al. (2006) used empirical models to predict mean krill growth rates simultaneously from food, temperature, length, sex and maturity stage for krill during the summer based on statistical relationships fitted to experimentally derived data.

These previous approaches, whilst accounting for the individual modification of physiological responses to modify growth, do not explicitly consider the mechanisms behind the growth rates modelled. They are phenomenological (statistical) models which simply fit lines to observed relationships between physiological and environmental variables and encounter problems of extrapolating outside the limits of these empirical functions. This non-mechanistic approach, in not including metabolic processes and the subsequent calculation of net production, limits future application because we have no knowledge of the environmental conditions under which the statistical relationship fails. Whilst they are useful for looking at observed conditions and timings, they are not suited to prediction and investigation of new regimes. The model described in this chapter is a mechanistic model, which starts from physiological first principles.

Physiologically based models have been developed for larval and non-feeding stages (Hofmann et al., 1992) and first feeding stage (Calyptopis I) to adult (Hofmann & Lascara, 2000; Alonzo & Mangel, 2001). The model implemented by Hofmann et al. (1992) considers developmental stages of the krill which are constrained energetically by not feeding and are not influenced by external environmental fluctuations (except temperature) to the same degree as adult stages. Hofmann and Lascara (2000) developed a physiological krill growth model, which was subsequently included in a Southern Ocean advection model by the work of Fach and colleagues (Fach et al., 2002; Fach, 2003). This

approach uses a time dependent, size-structured bioenergetically based model to estimate krill growth, parameterised by physiological data. However, the model takes no account of reproduction, or its associated costs, so whilst individual success in terms of growth can be assessed, it is not possible to infer reproductive capabilities from this study.

Hofmann and Lascara (2000) concluded from their model runs that where only pelagic phytoplankton was used as a food source, krill shrank during winter. They therefore added additional food sources and found that when a simulation for sea ice biota was added, krill grew realistically. Their model strongly suggests that krill are feeding during the winter. Krill showed a seasonal pattern of growth, with rapid growth in the spring and summer and minimal or no net growth in the winter. Fach (2003) also showed pelagic phytoplankton to be inadequate to support krill maintenance and growth during transport across the Scotia Sea, concluding that krill could barely sustain their initial size when feeding on phytoplankton as a sole food source, implying that larger krill need additional food sources during transport. Krill were seen to have improved survival when fed on additional food, or spent longer periods of time in the sea ice during transport. In both models, temperature had a positive effect on growth. The energetics model of Hofmann and Lascara (2000) included in Fach (2003) does not include growth of reproductive tissue, or spawning or moulting processes, unlike the model developed in this chapter.

Alonzo and Mangel (2001) also developed an energy budget model for krill, which takes the form of a dynamic state-variable model, considering the effect on the krill of three habitats, surface, shallow and deep water. Similar to the model of Hofmann and Lascara (2000), it is parameterised by experimentally gathered data and represents the traditional view of energy budgets, described in Equation 6.1. However, no account is taken of the cost of swimming, moulting or production of new tissue. Fecundity was considered, but was restricted to one spawning event a year, and this spawning had no

associated metabolic cost to the krill, was dependent on the size of the krill at a given point in time during the model run and was not influenced by environmental conditions.

It is clear that although significant progress has been made with the development of these models, we need to make considerable refinement to the structure and approach to modelling energy budgets in krill to adopt the concept of the energy budget proposed in Equation 6.2. Energetically based growth rate models are a powerful tool for testing various hypothesis on metabolic processes, and allow evaluation of the processes by which krill over winter (Hofmann & Lascara, 2000; Candy & Kawaguchi, 2006) and adapt to a changing environment.

This model presented here is based on the model devised by Hofmann and Lascara (2000), but developed by the adoption of the conceptual energetic principles discussed above (Equation 6.2). The objectives of this model are to define a structural, mechanistic model based on a sound physiological framework, following the energetic concepts outlined above, and thereby provide an accurate representation of a free-living krill in a natural habitat, allowing predictions about the animals life history to be made, and new insights in to physiology and behaviour and environmental responses gained. Additionally, by defining an energetic model with a robust structure, such as this, it allows for updated knowledge and experimental data to be incorporated to improve the models robustness when such advances are made, as well as serving to highlight where more concentrated experimentation in the field needs to be undertaken.

Choosing the state variables is the most crucial step in defining a system (Koojiman, 2000); the unit of currency in energy budget models varies between studies, and it is important to define what we mean by 'energy'. As discussed in Haynie (2001), energy is formally described as 'the ability to do work' which in a physiological context essentially means the ability to drive chemical reactions. Overcoming drag in swimming

for example, is doing mechanical work, but this mechanical work is achieved only by muscular activity, which is a process of converting chemical energy into mechanical work through a physiological (= chemical) reaction. Energy is stored in a collection of organic compounds so there is a close link between energy and mass flow. However, energy fluxes through living systems are difficult to measure (Koojiman, 2000), whereas measurements relating to mass are more straightforward. Growth involves the investment of energy, which is split between energy preserved in the new biomass, and that lost as heat during the synthesis of this new biomass. It is not practical to use size alone to represent mass, as the measure of the condition of an animal (in response to environmental conditions) reflects its chemical make up. For example, krill of the same body size may have different chemical composition depending on environmental variability or life history stage (see Chapter 3 for examples). Indeed, Färber-Lorda (1994) suggests that the environmental history of krill has a greater influence on wet mass and total length than does either sex or maturity stage.

This model uses carbon as its state variable, but many experimentally derived rates are based on length and/or mass, so it is necessary to find a means of converting between these units to allow calculations to be made in the model. Figure 6.1 shows the calculations used to convert between these units, described in detail later.

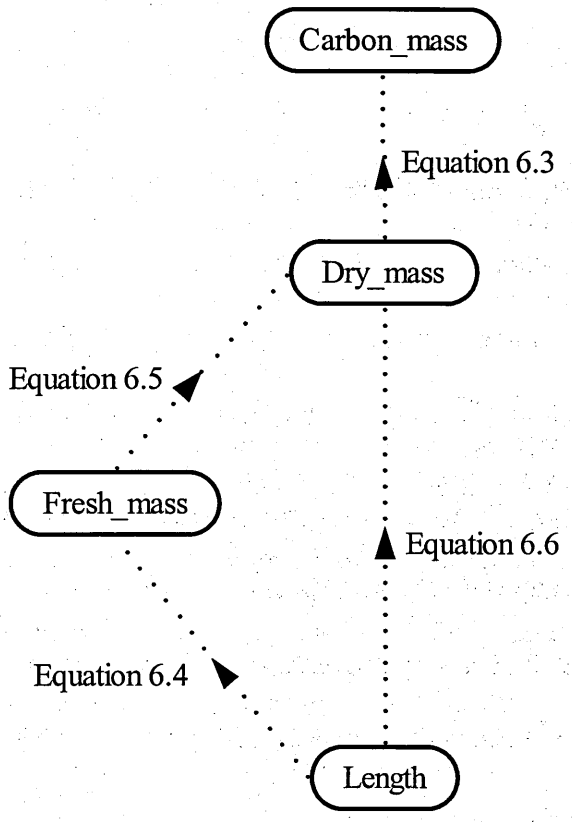

Figure 6.1. Schematic diagram of the conversions used to relate the descriptive units of measurement used in energetic studies, to carbon as used in the model.

The basic model structure used here takes account of the following components of krill physiology: feeding, metabolism, activity (swimming), reproduction and moulting and is influenced by the external environmental factors of temperature and food, as summarised in Figure 6.2.

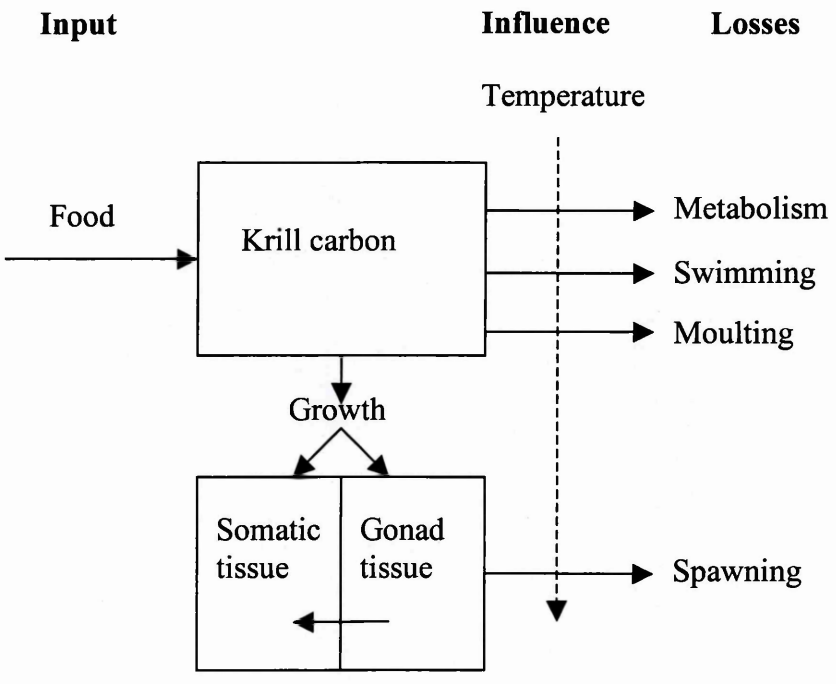

Figure 6.2. Summary of the key influences on the energetics of krill, shown as influences (temperature), inputs (food) and losses.

The key components of the model are the length/mass to carbon conversions (Figure 6.1) and the energetic model, which follows the energetic concepts discussed above (Equation 6.2). The outline structure of the energetic model is shown in Figure 6.3, with the component parts and their parameterisation discussed in the following section.

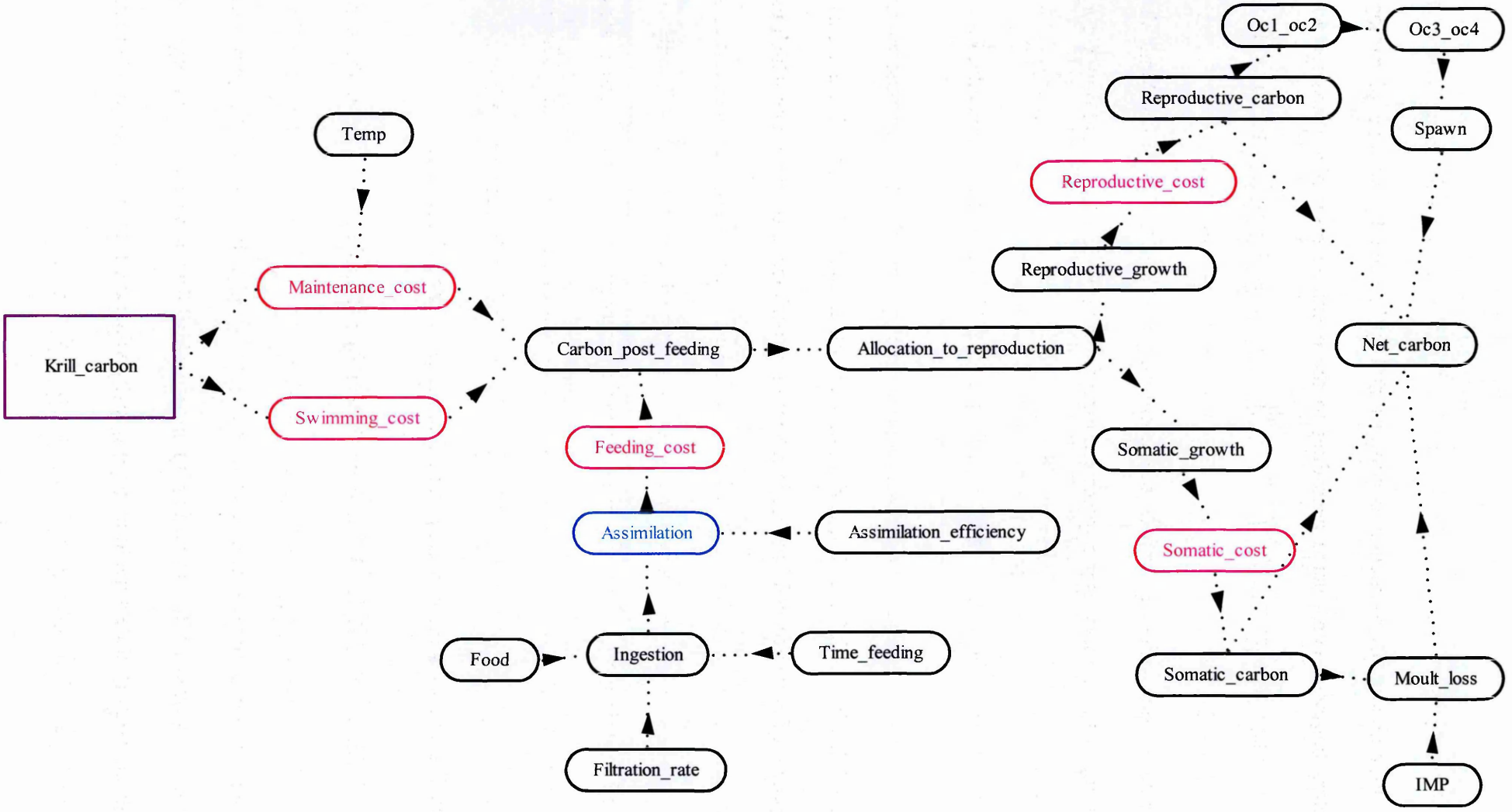

Figure 6.3. A simplified schematic of the energy budget model structure. Metabolic costs shown in red, carbon from feeding shown in blue.

MODEL DESCRIPTION

A time-dependant, energetically based deterministic model for an individual krill subject to realistic environmental conditions and influenced by various physiological parameters has been constructed according to the energetic principles discussed in the introduction to this chapter. This model is a dynamic model, developed as a continuous model in ModelMaker v4 (Cherwell Scientific Ltd.), then finalised and implemented as a discrete model run with one-day integration time steps in MATLAB v7 (The MathWorks, Inc.). Initially the model was parameterised for a female post-larval krill at South Georgia, run over time steps of a day, with $t_1 = 1^{st}$ January. Figure 6.3 shows as schematic of the model structure and the mathematical code for the model is presented in Appendix E.

An important choice is the most appropriate single variable to use as the currency in the model, whether it be joules, carbon or some other energetic unit. However, as the development of any successful model is reliant on experimental observations which use a range of units to express the results (for example length, dry mass, wet mass), it is also necessary to establish an accurate means of converting between these commonly used units. Since this model uses carbon as its main currency unit, we first need a step to convert from the more commonly used measurements of mass and length. The most accurate and logical first step would be to define a relationship between carbon and dry mass, as direct measurements were obtained in the experimental analysis.

As shown in Chapter 3, there was a significant difference between the carbon content of krill sampled during JR70 and JR82, so in order to derive a relationship between carbon and dry mass, the two cruises were treated separately. Initially, for the purposes of this model, data from JR70 was used as it represents krill from around South Georgia only, although the combined data could be used to extrapolate the model further to other areas. The relationship between carbon and dry mass derived was:

Equation 6.3

$$\text{Carbon mass (mg)} = 0.502455 \times \text{Dry mass (mg)} - 2.24989$$

as shown in Figure 6.4. This equation predicted a significantly higher carbon mass for a given dry mass than the relationship used by Hofmann and Lascara (2000), which was interpolated from a range of previously published data.

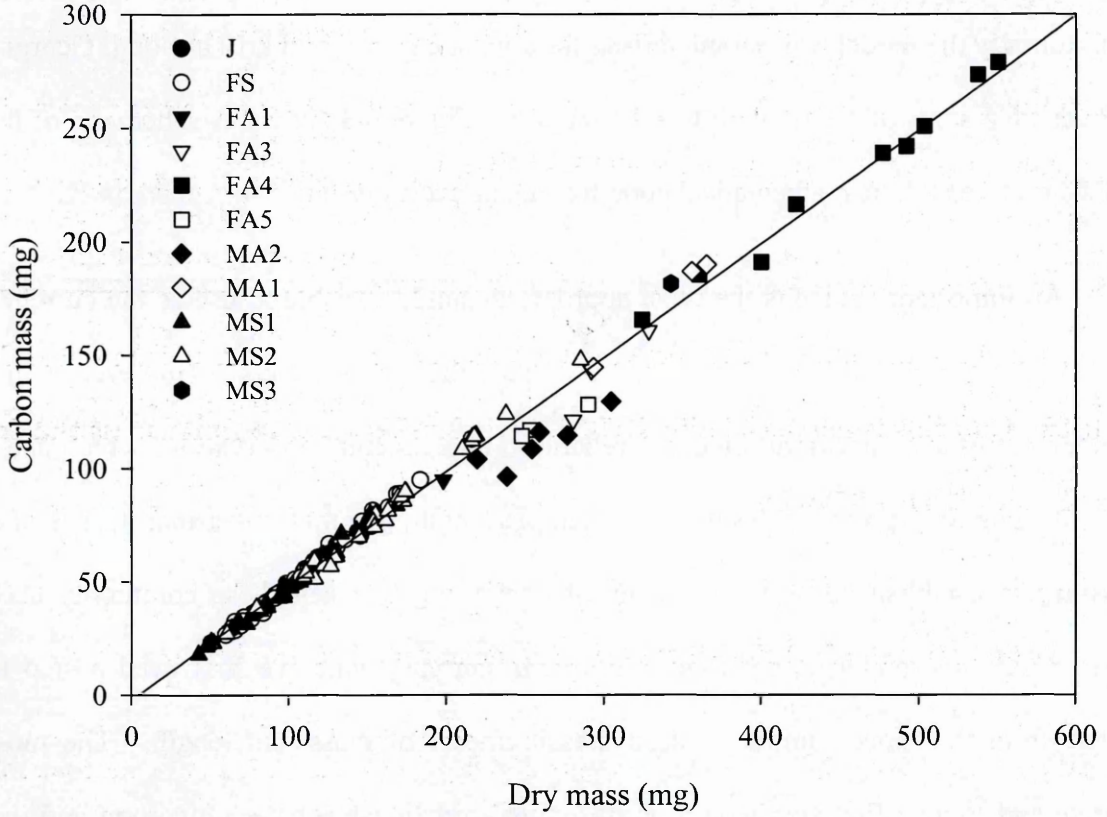

Figure 6.4. Relationship between dry mass and carbon mass for krill caught during JR70; $R^2 = 99\%$, $n = 158$.

There is a huge body of literature that presents length to mass conversions for *Euphausia superba*. Morris et al. (1988) discuss the merits of various methods of obtaining length-mass relationships and present a summary table of published relationships; an updated version of which is presented in Appendix B. Some authors when presenting model data have attempted to interpolate a range of length mass equations (Hofmann & Lascara, 2000), but face the problem of potential variation caused by

differing environmental conditions present in each study and differences in methods of describing length.

To parameterise the length to mass relationship, experimental data gathered by this study were used. In the case of the length to frozen mass (proxy for wet mass, as discussed in Chapter 2) relationships, there was no significant difference between samples from the two cruises (as discussed in Chapter 3), so the data were combined to give the following relationship ($R^2 = 96.6\%$, $n = 615$):

Equation 6.4

$$\text{Log}_{10}\,\text{Frozen mass (mg)} = -2.86494 + 3.4403\,\text{Log}_{10}\,\text{Length (mm)}$$

For the frozen mass to dry mass conversion, data from the cruises were treated separately as there was a significant difference between the water content of krill sampled from JR70 and JR82. For animals sampled during JR70, the relationship between frozen mass and dry mass ($R^2 = 94.9\%$, $n = 97$) was:

Equation 6.5

$$\text{Dry mass (mg)} = 5.61271 + 0.249439\,\text{Frozen mass (mg)}$$

In addition the relationship between dry mass and length was derived, shown in Equation 6.6 for JR70 krill (see Appendix B for relationships for JR82 and all krill pooled) ($R^2 = 94.3\%$, $n = 97$). Using a dry mass to length conversion removes one conversion step (see Figure 6.1), however, it was necessary to use the intermediate fresh mass calculation in the model for the purposes of comparing model run outputs with data from the literature, and it enabled the use of functions which were calculated from fresh mass to be used in the model where necessary. The difference in length estimations using the conversions used in the model (Equation 6.5 and Equation 6.4) and using dry mass to length directly (Equation 6.6) are shown in Table 6.1.

Equation 6.6

$$\text{Log}_{10}\text{ Dry mass (mg)} = -3.52004 + 3.48253\ \text{Log}_{10}\text{ Length (mm)}$$

Table 6.1. Difference in estimated lengths between using fresh mass as an intermediate step, as in the model (Equation 6.4) and converting directly from dry mass to length (Equation 6.6).

Length (mm) derived from Equation 6.4	Length (mm) derived from Equation 6.6	Percentage difference between estimations
22	24.14	9.74
25	26.57	6.27
30	30.91	3.04
35	35.48	1.36
40	40.16	0.39
45	44.90	0.22
50	49.68	0.63
55	54.49	0.93
60	59.31	1.16

There are drawbacks to using these morphometric conversions in the model. As each experimentally derived relationship contains variability, converting between units at each time step introduces an error. A fundamental problem in modelling the energetics of a growing, maturing krill not addressed by previous models, is that as it grows and matures, its morphometric characteristics change. Morris et al. (1988) showed that the length to mass relationship is dependant on the sexual maturity classification of the krill, and as I have shown in Chapter 3, the carbon and water contents also vary between animals of differing sexual maturity stages. Ideally, we would be able to track maturity stage developments in the model, and apply specific morphometric relationships depending on the maturity stage that would contain less variability. For example, a female krill of 55 mm would weigh significantly less if it were in stage FA2 compared to a heavily gravid FA4 krill of the same length. Also, carbon content would be significantly higher in a FA4 krill and water content significantly less. However, the problem of assigning boundaries for sexual maturity stage in terms of length or mass is a difficult one. Figure 6.5 (and Appendix F) show that there is considerable overlap in lengths between krill of different sexual maturity stages. In addition, varying environmental conditions may affect the rate

of maturation or growth; for example Watkins et al. (1999) showed the modal length of particular age classes in krill from South Georgia varies between years.

Reproductive development in krill is not a simple incremental process (Cuzin-Roudy & Amsler, 1991). Rather than an irreversible progression through maturity stages with age, krill reproduce several times in a season (Ross & Quetin, 1983; Cuzin-Roudy, 1987a), cycling through the adult maturity stages, and have the capacity to regress to a more juvenile stage following the reproductive season (Thomas & Ikeda, 1987) or periods of low food availability (Cuzin-Roudy, 2000). Therefore, it is clear that progression through the observed maturity stage classifications is not a one way process and there is no simple relationship between maturity stage and age.

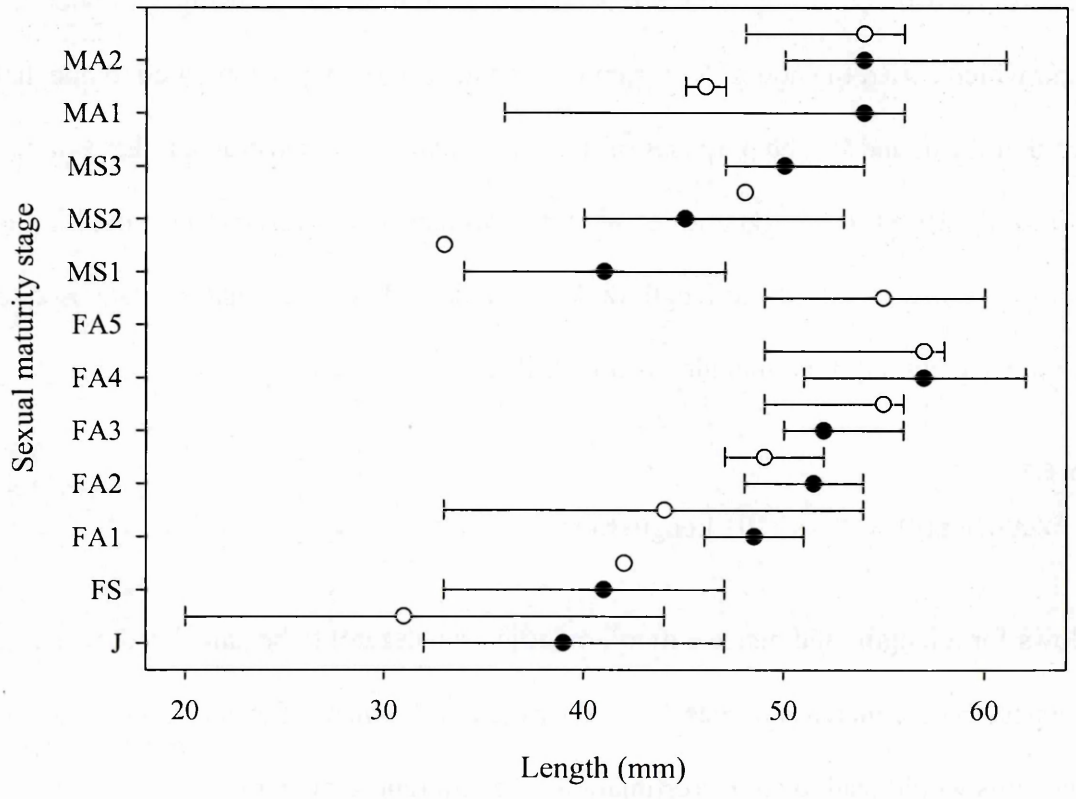

Figure 6.5 Range of lengths of krill from varying sexual maturity stages. Closed circles JR70 krill, open circles JR82 krill. Circles show median length, bars minimum and maximum values.

Because of the lack of clear morphometric boundaries between sexual maturity stages, this model does not track sexual maturity stage. However, if with further study such boundaries could be reliably defined, this would result in an improvement to the model in as much that it would represent what biologists measure in the field. Nevertheless, the model does attempt to allow for some of the variation in length/mass relationships with sexual maturity. The detailed study of Morris et al. (1988) explored the differences between combining all sexual maturity stages when considering length/mass regressions, and analysing separately. It was shown that sexual maturity stage significantly affects the nature of the length/mass relationship in krill, as might be expected when the difference between a gravid and non-gravid female of similar length is considered. Similarly, this affect of maturity stage on morphometric characteristics is highlighted in Figure 6.4, with a lower percentage carbon content in mature male krill. Because Morris et al. (1988) demonstrated a significant reduction in variation when using a model for regression which sorted the non-gravid from the gravid females when compared to one that considered all krill, and for the purposes of the way this model is constructed, the equation of Morris et al. (1988) for non-gravid female krill was used to convert carbon stored in the somatic tissue ('*somatic store*') to length (although it should be noted that the data used to establish this equation did not include juvenile krill):

Equation 6.7

$$\text{Wet mass (g)} = 10.88 \times 10^{6}\ \text{Length (mm)}^{2.9077}$$

This allows for a length (and hence a morphometric growth rate) to be calculated, which is not influenced by the increase in mass caused by the development of ovary tissue in gravid krill, since this would lead to an overestimation of length (and growth rate).

Parameterisation

Metabolism

Although there has been a large amount of previous work on the metabolic rate of krill, which shows that metabolic rate changes significantly with the size of animal and temperature (reviewed in Buchholz & Saborowski, 2000), we still lack fully robust estimates of metabolic rate. Summaries of the range of published oxygen consumption rates are given in Clarke and Morris (1983) and Quetin et al. (1994). The principle problems with deriving accurate estimates of metabolism experimentally are that methodology can affect the results and it is impossible to control for the effects of filtration rate, swimming activity and tissue synthesis during the measurement. These problems, of course, beset all zooplankton energetics work and are not peculiar to krill.

Basal metabolism is the cost to the animal of staying alive, and, as traditionally defined, does not include the costs incurred through activity or feeding. It represents the energy required for basal protein, lipid and nucleic acid maintenance, ion pump activity and basal circulation and nervous activity (Clarke, 1983). Many of the concepts underpinning the traditional approach to energetics, such as basal metabolic rate or specific dynamic action, originated from work on domesticated terrestrial mammals (Blaxter, 1967); they do not always translate easily to a continuously and actively swimming zooplankton like krill. To date it has not been possible to derive an accurate estimate of basal metabolism (as traditionally defined) for krill because as a free living planktonic organism, they have to swim continuously in order to survive and so any practical measurement of metabolism automatically includes a component associated with active swimming. It has been suggested that one way to solve this problem is to measure metabolic rates at differing swimming speeds and extrapolate back to find a theoretical metabolic rate at a swimming speed of zero (Clarke & Morris, 1983). Swadling et al. (2005) undertook a study to assess this. The relationship between swimming speed and

metabolic rate was not linear at lower swimming speeds, and hence the cost of swimming could not be easily discounted from metabolism.

In the model developed here, in the absence of any accurate assessments of basal metabolic activity, it was decided to use a relationship derived to quantify winter metabolic rates, published by Quetin and Ross (1991). This demonstrates a positive relationship between total length and metabolic rate using adults sampled during July and incubated individually in closed bottles at -1.5 °C (Equation 6.8). This relationship was chosen because in winter, post-larval krill are not actively feeding, growing or maturing gonads (or at least such processes are at a minimum), and hence winter metabolism is the best practical estimate we have of true basal metabolism in krill.

Equation 6.8

$$\mathrm{Log}_{10}\ VO_2\ (\mathrm{ml\ h^{-1}}) = 0.0285\ \mathrm{Length\ (mm)} - 2.7873$$

Winter metabolic rates were shown by Quetin and Ross (1991) to be only 33% of summer oxygen consumption. The authors hypothesised that while some of this reduction can be attributed to the fact that adult krill are not feeding in the winter, the remainder is likely to be due to other behavioural changes such as a reduction in swimming activity or to physiological cellular changes such as a decrease in metabolic enzyme activity. As such, this rate is currently considered to be the best approximation to basal metabolic rate (including swimming costs) available and differs from that used by Hofmann and Lascara (2000). As this measurement of metabolic rate does not control for swimming or filtration activity, it is defined in the model as '*maintenance cost*'.

Metabolic rates are converted in to mg C d^{-1} assuming that 0.5357 ml O_2 are consumed per mg carbon (Omori & Ikeda, 1984); this assumes a respiratory quotient (RQ) for protein metabolism of 1.0 (Ikeda & Dixon, 1982) and that all metabolism occurs using

protein as a substrate. The values 12/22.4 represent the mass of carbon (12 g) in 1 mole of carbon dioxide (22.4 litres).

Equation 6.9

$$\text{Carbon} = \text{Respiration Rate} \times \text{RQ} \times \frac{12}{22.4}$$

The assumption of an RQ of unity has been used previously in the models of Hofmann and Lascara (2000) and Huntley et al. (1994), deduced from observed oxygen to nitrogen ratios by Ikeda and Dixon (1982). The respiratory quotient (the molar ratio of carbon dioxide produced to oxygen consumed as a result of respiration (Omori & Ikeda, 1984)) varies between 0.7 and 1, depending on the metabolic substrate. Gnaiger (1983) showed that the RQ for respiration using lipid as a substrate is 0.72 and for protein it changes as a function of excretory product (i.e. 0.97 for ammonia and 0.84 for urea). The O : N ratio is known to be a good indicator of the respiratory metabolite in animals under starved conditions. When the ratio is higher than 24, the major metabolite is lipid, and when lower than 24, protein becomes the major metabolite, until the ratio reaches 8 which reflects the metabolism of solely protein (Ikeda, 1974; in Segawa et al., 1982). Segawa et al. (1982) recorded an O : N ratio of greater than 24, suggesting that lipid was a greater proportion of the metabolic substrate than protein. However, although the mean in this study was 28.29, individual ratios ranged from 8.26 to 95.42. It should be noted that these data were obtained under starved conditions, and krill in the field may utilise a different metabolic substrate in the presence of food. In general, marine zooplankton are considered to primarily excrete ammonia (Omori & Ikeda, 1984); the assumption of an RQ of one being consistent with this finding. Although the model was constructed on the assumption that RQ = unity, the sensitivity of the model to different RQ values was investigated, and the results are presented in Appendix G.

Temperature

Respiration rates are significantly affected by temperature (Clarke, 1987a). To correct the metabolic rate for the effects of changing water temperature a Q_{10} correction is applied; Q_{10} being the temperature coefficient which scales respiration rate with changing temperatures. It is defined as the factor by which respiration rate increases for a 10 °C rise in temperature. The range in temperature that Antarctic krill may potentially encounter during seasonal variation and regional transport (-2 to 5 °C) is large enough to potentially effect respiration rates (Quetin et al., 1994). For the purposes of this model, the scaling effect of temperature has only been applied to basal metabolism; although it is recognised that temperature will affect other metabolic processes similarly, we lack measures of their temperature sensitivity to apply to the model.

Several studies have investigated the effect of temperature on respiration, resulting in a range of published Q_{10} values. However, accurate data are limited as there have been many different methodologies applied to krill from different environmental water temperatures, at differing maturity stages, and it has been shown that Q_{10} values decrease with an increase in temperature (Rakusa-Suszczewski & Opalinski, 1978). Rakusa-Suszczewski and Opalinski (1978) observed the highest Q_{10} values in juveniles, but there was little difference between values obtained for male and female adult krill. A summary of published Q_{10} data for krill shows a range between 1.1 and 8.5, and is dependant upon the temperature at which it is derived (see Table 6.2). Comparative Q_{10} values from other euphausiid species include values around 2 for *E. pacifica* (2.11 (Small et al., 1966), 2.0 (Torres & Childress, 1983)) and 2.18 for a range of euphausiids and calanoid copepods (Ikeda & Hing Fay, 1981). It has been suggested (McWhinnie & Marciniak, 1964; Small et al., 1966) that Antarctic krill have a reduced Q_{10} when compared with Arctic species; a higher metabolic rate and a reduced Q_{10} provides *E. superba* with a mechanism to buffer against the effects of rapid or seasonal changes in environmental temperatures, however,

the data presented in Table 6.2 do not seem to wholly support this hypothesis, or that of a decrease with increasing temperatures (Rakusa-Suszczewski & Opalinski, 1978).

Table 6.2. Summary of published Q10 values from the literature for krill respiration rates, for a range of locations and temperatures. * outlying value, disregarded as unphysiological.

Study	Antarctic location	Date	Method	Sex	Temperature (°C)	Q_{10}
Rakusa-Suszczewski & Opalinski (1978)	South Georgia & The South Shetland Islands	Jan–March	Volumetric respirometer	Juvenile	-1.0–2.4	4.20
					1.5–2.4	1.25
				Male	-1.0–1.5	2.29
				Female	-1.0–1.5	2.22
McWhinnie & Marciniak (1964)	Bransfield Strait	Nov–March	Warburg technique	Adult	0–5	1.2
Segawa et al. (1979)	63-65° S 131-150° E	Jan	Winkler titration (Strickland & Parsons, 1972)	Large krill (43–52 mm)	-1.0–0	36.4*
					0–2	1.1
					2–3.5	1.1
					3.5–5	5.5
				Small krill (22–31 mm)	-1.0–0	2.1
					0–2	1.0
					2–3.5	3.0
					3.5–5	8.5
			Dissolved oxygen meter	Adult krill (32–38 mm)	-1.0–0	2.3
					0–2	2.1

Although there is variability in the results about the relationship with Q_{10} in relation to life history stage and temperature, with some unusually high values, the result are broadly consistent. As a Q_{10} correction was applied in the model to the metabolic rate described by Quetin and Ross (1991) for krill at -1.5 °C, an average value of 2.62 is calculated from the above table, including all data where the temperature range studied included -1 °C (with the omission of the obviously extreme asterisked value).

The Q_{10} correction is applied to metabolic rate using the following formula:

Equation 6.10

$$\text{Maintenance cost}_2 = \text{Maintenance cost}_1 \times Q_{10}^{\frac{T_2 - T_1}{10}}$$

where T_1 is -1.5 °C, the temperature winter respiration was measured at in the Quetin and Ross study, and T_2 the observed temperature at the particular time step in the model.

In the Southern Ocean, the temperature range is from -1.9 °C under the ice in winter to 5 °C in the summer at the northern limits of krill distribution. Within these temperature limits, several key aspects of the physiology of krill are strongly temperature dependent. Temperature affects development times of early larval stages, respiration rates and the intermoult period. Quetin et al. (1994) suggest that where there is enough quantitative data, temperature effects appear to be more pronounced than would be expected from a typical Q_{10} of 2–3. There is also some evidence that for some physiological processes e.g. larval development, Q_{10} values rise steeply at the very lowest temperatures. Nevertheless for ease of computation, a single Q_{10} value was assumed, and based on the data in Table 6.2, this was set at 2.62.

The temperature field in the model was initialised with a data series for South Georgia obtained from the IRI/LDEO Climate Data Library website, (http://ingrid.ldeo.columbia.edu/). Data were extracted from the NOAA NCEP EMC CMB global optimum interpolation sea surface temperature analysis (version 2), which provides worldwide weekly means on a one degree grid scale. Data were extracted over the available range of dates (1981–2004), for a one degree grid centred on 54.5° S, 39.5° W (which represents the centre of the Western Core Box sampling site, north west of South Georgia) as shown in Figure 6.6. An overall mean yearly trend was estimated using the mean value for each week for all years sampled to produce the generalised temperature curve for South Georgia, shown in Figure 6.7. This temperature curve was entered into MATLAB and a spline interpolation fitted to generate temperate values on a daily, rather than weekly basis.

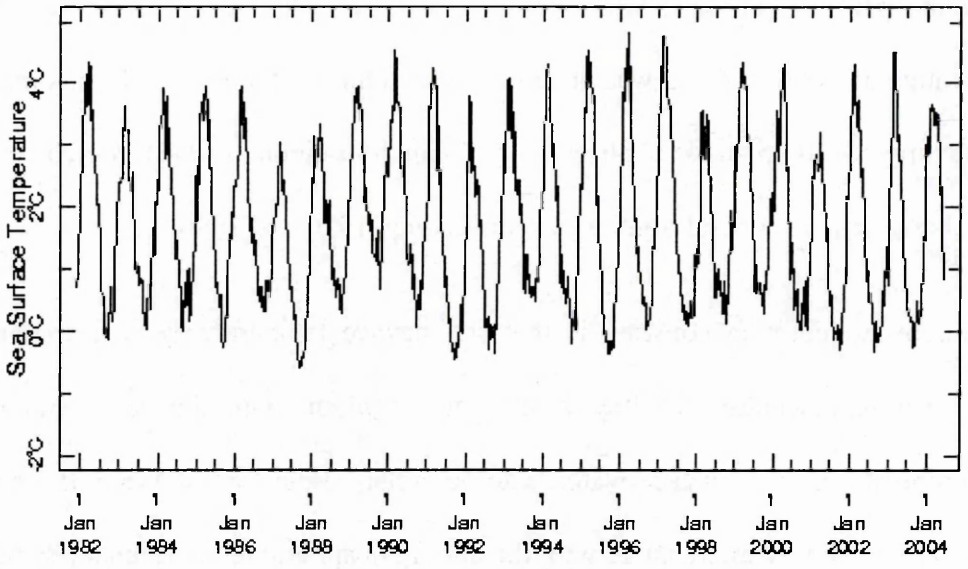

Figure 6.6. Variation in annual temperature ranges for South Georgia between 1981 and 2004. Data as extracted from the NOAA NCEP EMC CMB (National Oceanic and Atmospheric Administration, National Centres for Environmental Prediction Environmental Modelling Centre Climate Modelling Branch) global optimum interpolation sea surface temperature analysis (using Global SST data from Reynolds & Smith, 1995), with weekly data points. These data are used to produce the weekly mean temperatures used in the model (as shown in Figure 6.7.)

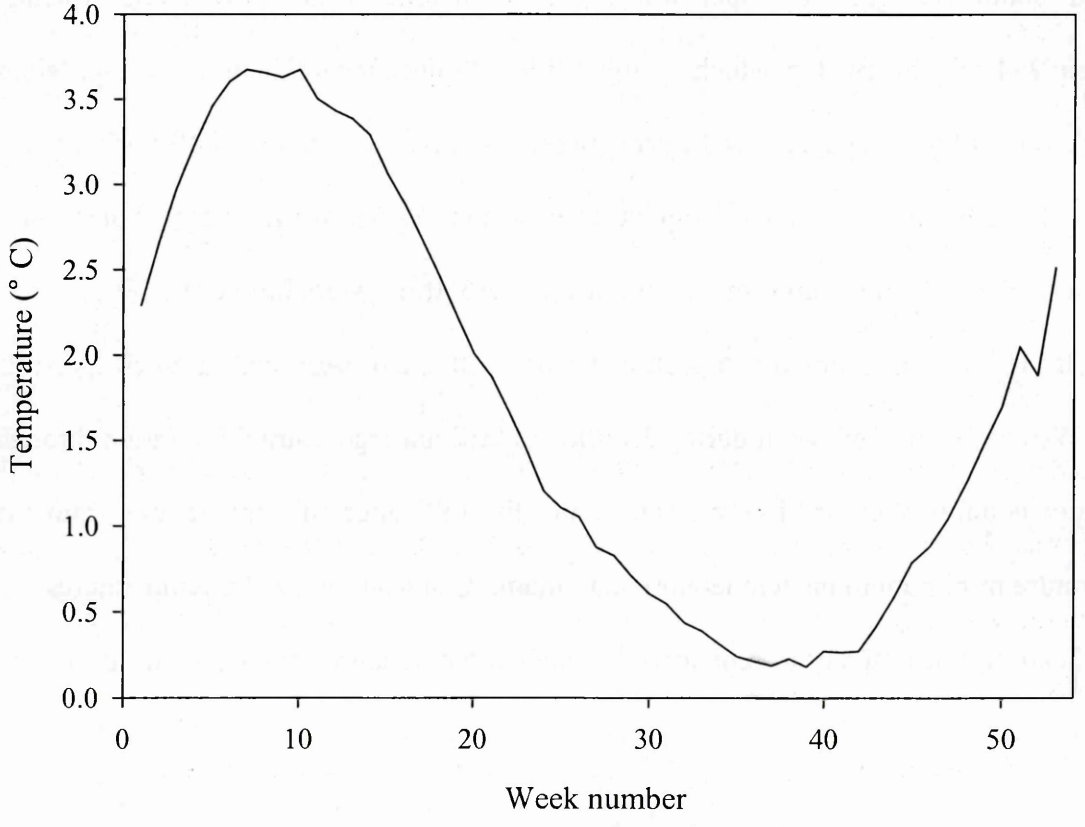

Figure 6.7. Mean weekly sea surface temperature for 1981-2004 for South Georgia.

Over the period data was collected for (December 1981 to December 2004), a temperature range of -1.06–4.76 °C was observed, with a mean of 1.73 °C. This compares well with the range of -0.95–4.44 °C observed by Whitehouse et al. (1996) in their review of seawater characteristics around South Georgia between 1926 and 1990.

An important point to consider is that sea surface temperatures measured from satellites are not representative of the whole water column, with the stratified layers varying in temperature. The surface waters can be either colder than those of the mixed layer, as they are cooled by interchange with the cool atmospheric temperatures (Barnes et al., in press) or warmer where solar radiation raises the temperature. More stable, low temperatures are found in the deep layer at around 100m, which can be significantly cooler than the surface waters. However, seasonal mixing can alter the stratification of water masses, and north of the Southern Boundary there is less stratification in the water column. Around South Georgia, the upper water mass (Antarctic Surface Water) is typically between 2–4 °C, below the which is the Winter Water (the remnant of the previous winter's mixed layer), characterised by a temperature minimum at around 100–150 m, and is typically between -1–1 °C. Circumpolar Deep Water lies below the Winter Water and is characterised by a temperature maximum at around 500 m (Meredith et al., 2005). (See Appendix H for temperature depth profiles for on-shelf, shelf break and off-shelf transects of the Western Core Box taken during JR70). As krill undergo diurnal migration through the water column, they are likely to encounter the full range of temperatures from the temperature minimum to the temperature maximum. Using surface water temperatures is a simplification, but is likely to represent the median temperatures encountered during any one day.

Swimming

As described previously, our ability to construct accurate energy budgets for krill have been hampered by our lack of knowledge of the metabolic costs associated with

swimming (Quetin et al., 1994; Swadling et al., 2005). Krill must swim continuously in order to maintain their position in the water column; this activity has an associated metabolic cost to the animal, which is included in the model as '*swimming cost*'. It is important to consider this metabolic cost separately in terms of a correct energy budget. It has been shown that there is an increase in metabolic rate with increasing swimming speeds (Swadling et al., 2005), with a linear increase in oxygen consumption at increased swimming speeds of 5 cm s^{-1} or above. A swimming speed of 3 cm s^{-1}, although perfectly adequate for the krill to maintain its position in the water column, was not sufficient to induce these elevated levels of oxygen consumption. This complex relationship between swimming speed and oxygen consumption means that it is not possible to estimate basal metabolism independent of swimming costs in krill. Kils (1981) made an estimation of the metabolic cost to a krill of maintaining its position in the water column, by comparing data for krill with measurements taken on the primarily benthic shrimp *Crangon crangon*. This gave a metabolic cost of holding station in the water column (which Kils (1981) termed hovering) of 0.77 mg O_2 dry mass (g) $^{-1}$ hour $^{-1}$ (equivalent to between 2.5% body carbon for a 22 mm krill and 2% for a 60 mm krill). Although it was assumed that a krill would be a more efficient swimmer than the benthic shrimp, the method for measuring *C. crangon* was considered to be an underestimate, and Kils (1981) hypothesised that the true value for krill would be only slightly lower than the above estimate. However, when trialled in the model, it was seen that, compared with the other energetic parameters, this assessment of the cost of swimming was high, leading to negative growth in large krill under average food conditions (see Appendix G, Table 2). From hydrodynamic considerations Kils (1981) concluded that it is more energy-efficient for krill to swim at speeds between 3 and 10 cm s^{-1} than to hover in one place, however this is in conflict with results of Swadling et al. (2005). In a separate calculation, Kils (1981) estimated that a 60 mm krill requires a carbon input of 0.0054 mg C mg dry body mass $^{-1}$ day $^{-1}$ in order to

hover in one position; equivalent to 0.5% body carbon. Therefore, to initialise the model, '*swimming cost*' was parameterised as 0.5% of total body carbon, although this is likely to be an underestimate for periods of very active swimming.

Feeding

The primary mode by which krill feed is by active filtering of the water column for available food, such as pelagic phytoplankton or microzooplankton (Marr, 1962; Quetin & Ross, 1985; Schnack, 1985). Filtration rates for krill have been estimated in numerous laboratory experiments and appear to be dependant on the size of krill (reviewed by (Morris & Priddle (1984), Daly (1990), Quetin & Ross (1985)), and to some extent, particle size. Not all particles are filtered with equal efficiency and some form of particle selectivity does occur (Morris, 1984). Morris (1984) presented a review of previous studies of filtration rate available at the time and concluded that many of the early published values for filtration rate were significant underestimates. This was because in traditional small, closed volume filtration experiments most of the particles are initially removed more rapidly than the sampling interval can record. By increasing the size of the container in such experiments the estimated filtration rate was increased (Price et al., 1988), but still results in values significantly lower than those calculated by field ingestion rates (Quetin et al., 1994).

In the model developed by Hofmann and Lascara (2000), reliable data from a range of experimental studies (Morris, 1984; Quetin & Ross, 1985; Schnack, 1985; Daly, 1990; Quetin et al., 1994) were used to derive a relationship between krill dry mass and compression filtration rate. The equation derived by Hofmann and Lascara (2000) has been used to represent the filtration rate in this study, and is shown in Figure 6.8.

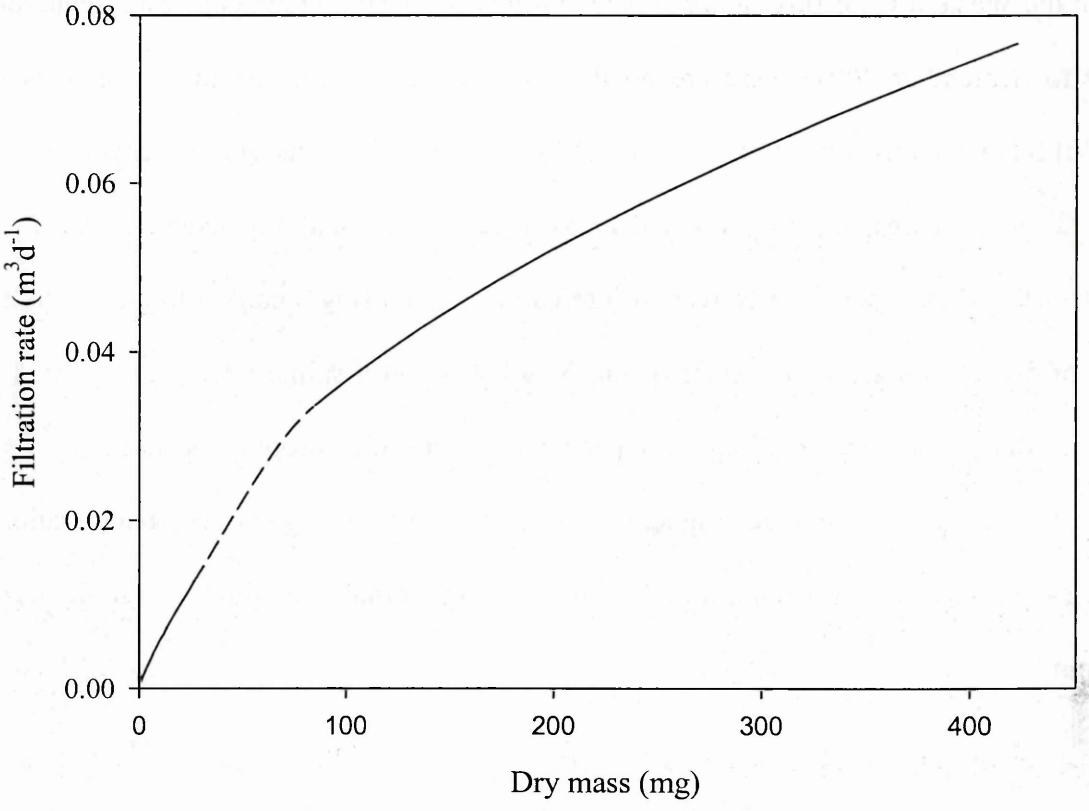

Figure 6.8. Filtration rate as used in the model. Solid lines represent the equations for filtration rate defined by Hofmann and Lascara (2000) for krill <26 mg and >84 mg dry mass (DM). Dashed line represents the spline interpolation, as calculated in the model runs for intermediate dry masses. For krill <26 mg DM filtration rate = 0.00085 $DM^{0.825}$, for krill >84 mg DM = 0.00343 $DM^{0.514}$.

Although laboratory studies by Morris et al. (1983) showed that krill kept in the laboratory feed throughout a 24 hour period, it is likely that in the wild animals do not feed all of the time. Food is patchy, and there are occasions when the krill will be travelling between patches of food, or undergoing diurnal migration when the rate of filtration is reduced. A parameter, '*time feeding*' was therefore introduced into the model to account for the percentage of a day during which the animal is undergoing active feeding, and was initially set at 75% for post-larval krill (Hofmann & Lascara, 2000).

The food available to krill was initially assumed to only be the phytoplankton present in the water column. Data on phytoplankton concentrations was obtained from SeaWiFS-derived estimates of surface chlorophyll *a* concentrations gathered from the Goddard Distributed Active Archive Centre at a weekly (8 day), 9 km resolution and

processed with the SeaDAS software. Data were collected over an area 53–54° S, 37–38° W (in the Western Core Box sampling site, north west of South Georgia) from September 1999 to September 2004 (there are no data for June and some of July as there is no SeaWiFS coverage at this time). Weekly mean chlorophyll concentrations were calculated over all grid squares, for all years and an average annual trend calculated, as shown in Figure 6.9. These data were converted into carbon units using a carbon to chlorophyll *a* ratio of 50 (De Jonge, 1980), entered into MATLAB and a spline interpolation fitted to generate daily food concentrations. The peak abundances of chlorophyll occur in the upper 2.5 m of the water column (Kawaguchi et al., 1986), so by using surface concentrations, these can be seen as a maximum for a krill undergoing diurnal migration through the water column.

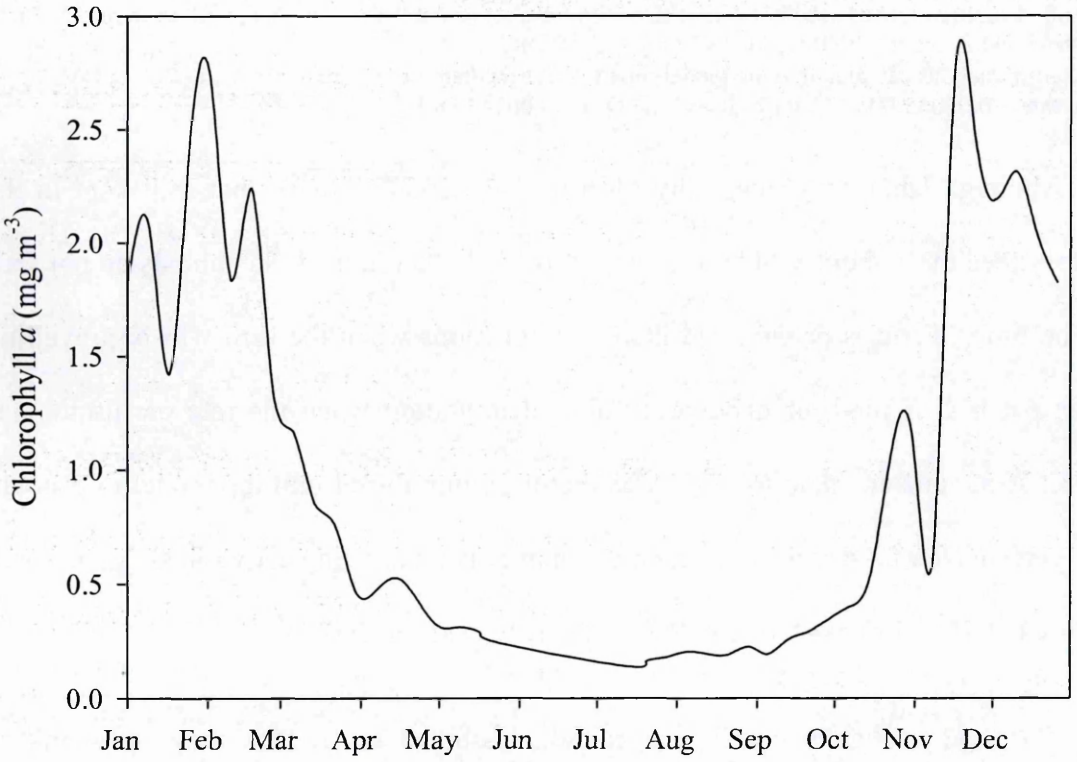

Figure 6.9. Weekly mean chlorophyll *a* concentrations derived for South Georgia between 1999–2004.

These data provide mean chlorophyll concentrations, averaged over time and space, producing a relatively smooth trend of higher chlorophyll in the summer, and low levels in

the winter. In reality, however, chlorophyll is patchily distributed, and may occur in patches of a very high concentration, next to areas of almost no phytoplankton. Indeed, the range of values obtained by SeaWiFS for this area was 0.01–64.6 mg m^{-3}. Although the maximum concentration is extreme, and may result from an error in the sensing technique, values between 10–30 mg m^{-3} for individual grid squares are frequently recorded during the summer, especially close to shore. By comparison a range of 0.12–26.83 mg m^{-3} for chlorophyll *a* concentrations around South Georgia between 1981 and 1990 was detected with direct ship based techniques (Whitehouse et al., 1996). Korb et al. (2004) examined the correlation between *in situ* ship based estimations of chlorophyll *a* and SeaWiFS satellite estimations around South Georgia and found despite a significant correlation between the two data sets, SeaWiFS underestimated the chlorophyll concentrations relative to ship board measurements, especially in areas of high primary production (where SeaWiFS estimations were up to 30% of *in situ* values).

Assimilation efficiency is the efficiency with which krill process the food that they collect during the process of feeding. This takes into account losses during feeding attributed to 'messy eating' and losses during the process of digestion. Kato et al. (1982) reported assimilation efficiencies for *E. superba* fed on a mixture of microalgae, ranging from 71.9–94.6% and Schnack (1985) observed a range of 70.4–83.4% for krill fed on two diatom species. Morris (1985) calculated an assimilation efficiency of 89% from egestion rates and estimated energy requirements, although he hypothesized that this value could be somewhat elevated due to potential low egestion rates for krill kept in the laboratory. A value of 80% was adopted for this study, as it falls in the middle of these published ranges.

All feeding activity has an associated metabolic cost; this comprises the mechanical action of filtration and the digestion of ingested food, both of which require energy. This cost of feeding has previously been described as specific dynamic action (SDA) (Jobling, 1983; Thor, 2002) which refers to the post-prandial rise in metabolic rate (McCue, in

press). However, Jobling (1983) showed that SDA equals the cost of moving food along the gut plus the costs of digestion/absorption and the cost of growth, where the cost of growth predominates. So the estimate used here for *'feeding cost'* (essentially SDA – cost of growth) is 1% of metabolic rate, although it is suspected that the true value will be much higher.

Moulting

Krill have to moult in order to grow, but unlike many species of crustacean, krill moult regularly regardless of whether they are growing or not. The periodicity of these moulting events is known as the intermoult period (IMP). An early review of moulting was undertaken by Clarke and Morris (1983) but much work has been undertaken since; Buchholz has provided much of the definitive work on euphausiid moulting and conducted a more recent review on moulting and growth (Buchholz, 1991). Moulting has been examined in both the laboratory (Murano et al., 1979; Nicol & Stolp, 1990; Buchholz, 1991) and in the field (Morris & Priddle, 1984; Buchholz et al., 1989; Shreeve et al., 2005). It has been shown that IMP varies in relation to temperature, with longer IMPs at lower temperatures (Buchholz, 1991; Quetin et al., 1994). Food availability and size of the krill also has an effect on IMP, but this has not been quantified in a way that allows predicative estimations of IMP.

In the model, IMP is dependent on temperature, according to the relationship as published in Buchholz (1991), with longer IMPs in the winter.

Equation 6.11

$$\text{IMP} = (\text{Temperature } (^\circ\text{C}) \times -3.04) + 23.21$$

As such, the IMP is changing continuously with time, driven by the temperature field. In order to keep track of the changing IMP and to allow the triggering of the moult and associated cost in the model, the index, z, is used to count time, being reset to zero at each

moult. This allows IMP to be evaluated against the time passed since the last moult at each step in the model run.

Kato et al. (1982) reported that krill maintained in a laboratory and fed on microalgae lost 2.05% of body carbon through moulting (range 1.4–2.5%) and Clarke and Morris (1983) calculated a value of 1.58% carbon lost at moult. As well as the carbon loss in the exuviae, there is potentially a metabolic cost included in the action of moulting, but this is included in the measurements of Kato so the value of 2.05% is used to parameterise '*moult loss*' in the model.

Reproduction

If there is enough food to provide additional energy after the costs of maintenance are met, then the krill uses this energy for growth and maturation. First the animal has to grow in length and develop the reproductive structures to enable it to be sexually mature. In the model it is assumed that once the krill has attained a predetermined size, it can begin to invest some of this extra energy into reproduction. Allocating a threshold size at which the animal becomes sexually mature is difficult, as this depends, presumably, upon the environmental conditions under which the krill is developing. This point of reproductive maturity has been introduced in to the model as the parameter '*start reproduction*'. As the krill in this model are female, the initial value for reproductive maturity was parameterised as the smallest observed krill of maturity stage FA1; this is the point at which krill have fully developed ovarian structures, and are ready to start producing oocytes (Cuzin-Roudy, 1987b). Appendix F shows the sizes of krill caught during JR70 and JR82, grouped by maturity stage and compared with the range of sizes of krill measured and classified in the same manner by Morris et al. (1988). During JR70, very few krill of stage FA1 were observed, and only 2 were analysed in this study, but the minimum size was 46 mm. During JR80 many more FA1 stage krill were encountered, 33 of which were measured, indicating a much smaller minimum size of 33 mm. Much larger numbers of FA1 were

measured by the Morris et al. (1988) study and their minimum size observation was 37 mm. Initially, the model was parameterised using the smallest of these observed lengths, 33 mm, which was converted in to carbon units using the previously described morphometric conversions. Cuzin-Roudy (1987b) states that spawning first occurs when krill are approximately 33 mm in length, so these estimates of minimum size seem to be broadly in agreement. If the animals are investing most of the energy into developing oocytes and not into growth at this point, and considering that it is likely that krill start to reproduce at smaller sizes in areas of reduced food availability. These observations suggest that the minimum size of reproduction in krill is less than the 38 mm suggested by Mauchline (1980a).

If the size of the krill at a particular time step in the model is greater than the value for '*start reproduction*', then energy is allocated to the production and development of oocytes. The proportion of available energy allocated to reproduction in krill is unknown, but we hypothesise that in times of plentiful food, the animal maximises the opportunity to reproduce and channels 70% of available resources into reproduction, parameterised in the model as '*allocation to reproduction*'. A review of the literature for a wide range of animal species, Koojiman (2000) concluded that a value of 70% available energy partitioned to reproduction is the most appropriate general parameter across species.

After available carbon has been partitioned into that available for somatic growth and reproductive growth (tracked in '*somatic growth*' and '*reproductive growth*'), the metabolic cost of synthesising this tissues is subtracted ('*cost of growth*'). There is an energetic cost associated with the synthesis of new tissue, which can be defined as 'the heat production which is due to biosynthesis' (Parry, 1983) (and it comprises the major part of SDA (Jobling, 1983)). In order to estimate the cost of growth for animal tissue, Parry (1983) calculated the theoretical efficiency of synthesis for a range of biochemical components, using a range of substrates common to ectotherms. This efficiency of

synthesis ranged from 70–98%. However, he demonstrated that empirical estimates were generally lower than the theoretical estimates of growth efficiency, with most values varying between 60–80%. An intermediate value of 70% was therefore taken, which equates to a cost of growth for a typical ectotherm of 30%, which is used in the model in the absence of a value calculated or measured specifically for krill.

Fecundity is technically difficult to estimate as krill release eggs directly into the water column in multiple spawning events (Cuzin-Roudy, 2000) and both brood size and periodicity of multiple spawns are still debated. Fecundity fluctuates with year to year variation in *E. superba* in the duration of the reproductive period depending on the extent of ice cover and food availability (Cuzin-Roudy, 2000). When the seasonal sea ice melting happens fast, the observed spawning season of krill starts early and is intensive, as during melting the stratification conditions favour phytoplankton development allowing krill to feed well and complete maturation quickly. When seasonal melting of ice is slow (in 'cold years') intense phytoplankton development is prevented, and krill mature more slowly leading to a later start of the reproductive season (Spiridonov, 1995). In productive regions, krill spawn high numbers of eggs frequently, compared with areas of low productivity where krill may spawn only a few batches of eggs, or none (Quetin & Ross, 1991).

As we are concerned with an energetic model rather than that of population growth in the first instance, the model is concerned not with fecundity (number of eggs produced), but rather with the energetic investment in the development of the ovaries, and subsequent loss at spawning. As such, it is important to model the development of the ovary throughout the reproductive season accurately. Females from differing sampling locations and dates have differing sexual development stages (which we hypothesise is linked to available food and temperature range) and large females produce relatively much larger egg batches than small females.

The structure of a developing ovary is complex, containing up to four groups of oocytes at different stages of development:

1. Young oocytes and oocytes accumulating glycoproteic yolk (previtellogenesis) are present in the ovary throughout the reproductive season. (oc1 and oc2 oocytes).

2. Oocytes in the phase of lipid yolk accumulation (vitellogeneisis) or already maturing (in meiosis) and mature oocytes form the next batch to be released (oc3 and oc4 oocytes).

3. The mature egg batch is spawned but the ovary is not emptied, the most advanced previtellogenic oocytes have already started vitellogenesis for the preparation of a new egg batch. (Cuzin-Roudy, 2000).

Because of this important division in the ovary between developing and maturing oocytes, the ovary of the krill in the model is divided into two sections, '*oc1 oc2*' and '*oc3 oc4*'. The developing oocyte compartment (*oc1 oc2*) must reach a certain threshold before the mature oocytes are produced. The parameterisation of this division between these two compartments is complicated, as the total number of developing oocytes (oc1–oc4) is proportional to the wet mass of the ovary, yet the number of previtellogenic oocytes is negatively related to ovary mass; large females have smaller stock of previtellogenic oocytes than small females (Cuzin-Roudy, 2000). In addition, we must first define the size of the whole ovary, in relation to the size of the krill at any given time step. There have been a number of studies which have attempted to define the size of the ovaries in mature krill, either by number of eggs (Denys & McWhinnie, 1982; Ross & Quetin, 1983; Harrington & Ikeda, 1986; Nicol et al., 1995) or by the mass or volume of the ovary (Mauchline, 1968; Cuzin-Roudy & Amsler, 1991; Quetin & Ross, 2001) and an attempt has been made to summarise some of these results, and estimate the percentage of total body carbon represented by the ovary, as calculated by each method (Table 6.3).

Calculations in Table 6.3 use experimental analysis of the C, H, N and water content of exercised ovaries (methodology described in Chapter 3), which gave an average value of 73.67% water (as fresh mass) and 54.84% carbon (as dry mass) for ovary tissue from FA4 krill of a range of 51–62 mm sampled during JR70.

Table 6.3. Size of ovary. All mass values given in mg. Ovary tissue is 54.84% carbon (DM) from CHN analysis, 73.67% water (FM)*. Body carbon value for krill 45 mm = 83.89, for 60 mm = 224.74.

	45 mm				60 mm			
Study	Dry mass	Wet mass	C mass	% body C	Dry mass	Wet mass	C mass	% body C
Nicol et al. (1995) [1]	84.48	156.78	46.33	55.23	195.01	380.06	106.95	47.59
Nicol et al. (1995) [2]	84.78	152.91	46.49	55.42	195.70	370.69	107.32	47.75
Cuzin spherical vol	40.00	151.91	21.94	26.15	1989.14	7554.66	1090.85	485
Clarke &	116.83	443.7 [3]	64.07	50.89	184.31	700.00 [4]	101.08	50.11
Morris (1983)	233.63	887.3 [5]	128.12	50.99	350.45	1331.0 [6]	192.19	51.04

1. average mass loss between gravid and spent females as a function of length
2. mean difference between spent and gravid masses as a percentage (34% dry mass, 17% wet mass)
3. Fresh mass 1 g
4. Fresh mass 1.605 g
5. Fresh mass 2g
6. Fresh mass 3g

Direct estimates of the size of an ovary have been undertaken: the study of Nicol et al. (1995) used both the average mass loss between gravid and spent females as a function of length, and the mean difference between spent and gravid masses as a percentage (34% dry mass, 17% wet mass) to estimate loss on spawning. Ovary mass can be estimated from the number of eggs per spawn or per ovary as given in various published studies. A given diameter for the range of maturity stages in the oocycytes is published in Cuzin-Roudy (2000), from which a spherical volume can be approximated and the volume of the spawned eggs calculated. If the assumption is made that the oocytes have the equivalent mass as water (whereby mass = volume in SI units) then the calculation is straightforward. However, it is likely that this is a slight overestimate (from experimental data ovary tissue is 73.57% water), with the remainder likely to be lipid, which is less dense than water.

Cuzin-Roudy (2000) derived a relationship between body mass and number of oocytes in an ovary, to which this spherical volume was applied.

With the exception of spherical volume data, Table 6.3 shows general agreement that the ovary of a mature krill represents approximately 50% of its total carbon content. The initial division between previtellogenic and mature oocytes was also assumed to be 50%, as although a relationship between size and number of mature oocytes was published in Cuzin-Roudy (2000), it was not applicable over the large range of sizes required for this model. Therefore, the parameter '*spawn*' was created to represent the division between (*oc1 and oc2*) and (*oc3 andoc4*) partitions of the ovary. Spawn has been parameterised as 25% of total krill carbon (where 50% of the ovary is each type of oocytes, and the whole ovary is 50% of total body carbon).

Spawning of mature oocytes does not occur at the time of moulting itself (i.e. ecdysis), rather they appear to spawn to coincide with apolysis (when the epidermis resumes its activity for preparation of the next moult), typically about 5 days prior to the next moult, depending on IMP (Cuzin-Roudy, 2000). Two successive moult cycles were necessary for a complete cycle of egg production, therefore spawning of mature oocytes was set to occur at every second moult cycle minus 5 days (i.e. 2 x IMP minus 5 days). The moult cycle is temperature dependent, but is typically around 20 days, and never below 9 days. In order to keep track of the changing potential for spawning, the index y, is used to count time, reset to zero/empty at each spawning event and allowing for the changing IMP. Previous IMP is stored in the model as a constant '*Prev_IMP*', and added to the current IMP at each time step in the model, minus 5 days and evaluated against y and time. This spawning event is allowed to happen, regardless of the content of the mature oocyte compartment.

Winter adjustments

In the winter, as food levels are lower, any remaining oocytes in the ovary are reabsorbed slowly (Denys & McWhinnie, 1982) to provide energy to the animal in a time of scarce food. The parameter '*reproductive decay*' is defined as the rate at which this tissue is reabsorbed by the animal, and is set at an arbitrary rate of 2% a day. The period of winter was defined as May to mid August (day 120–220). In terms of the model, carbon is reabsorbed directly into the somatic tissue from the reproductive tissue, but in reality it is likely that this reabsorption would involve the transfer of nutrients in the haemolymph to the mid-gut gland, where storage and much metabolism takes place. In the model of Hofmann and Lascara (2000) they introduced seasonal adjustments in a more deterministic way, using a seasonal activity factor to scale respiration down during winter to 50% of the summer value. In contrast in this model, respiration was summed from individual costs.

Table 6.4. Parameter definitions, units and initial values used in the krill energy budget model, where day one in the model is 1st January and krill length at day one = 22 mm.

Model element	Description	Units	Definition	Initial value	Parameter/ variable
Krill_carbon	Total body carbon mass	mg C		7.6747	variable
Temperature	Sea surface temperature	°C	Figure 6.7	2.2887	variable
Food	Phytoplankton concentration	mg C m^{-3}	Figure 6.9	91.1077	variable
Dry_mass	Krill dry mass	mg	Equation 6.3	19.7522	variable
Wet_mass	Krill wet mass	mg	Equation 6.5	56.6851	variable
Length	Krill body length	mm	Equation 6.4	22	variable
Maintenance_cost	Basal metabolism	mg C d^{-1}	Equation 6.8	0.1280	variable
Q_{10}	Temperature coefficient of respiration	—	2.62	2.62	parameter
Swimming_cost	Metabolic cost of swimming	mg C d^{-1}	0.5% body C	0.0384	variable
Filtration_rate	Rate krill filters a known volume of seawater	m^3 d^{-1}	Figure 6.8	0.0100	variable
Time_feeding	Time spent feeding	% (day)	75	0.75	variable
Assimilation_efficiency	Assimilation efficiency	%	80	0.80	parameter
Feeding_cost	Metabolic cost of feeding	mg C d^{-1}	1% metabolic rate	0.00096	variable
Start_repro	Threshold above which krill are mature enough to reproduce	mg C	33 (mm)	29.2295	parameter
Allocation_to_reproduction	Allocation to reproduction	% (C)	70	0.70	parameter
Cost_of_growth	Metabolic cost of synthesising new tissue	% (C)	30	0.30	parameter
IMP	Inter moult period	days	Equation 6.11	16.2524	variable
Moult_loss	Carbon loss at moulting	mg C	2.05% body C	0	variable
Spawn	Threshold for ovary size	mg C	25% body C	1.9187	parameter
Reproductive_decay	Rate at which ovarian tissue is re-absorbed in winter	% (per day)	2	0.02	parameter
Net_carbon	∂ carbon/∂ time	mg C	—	0.2640	variable

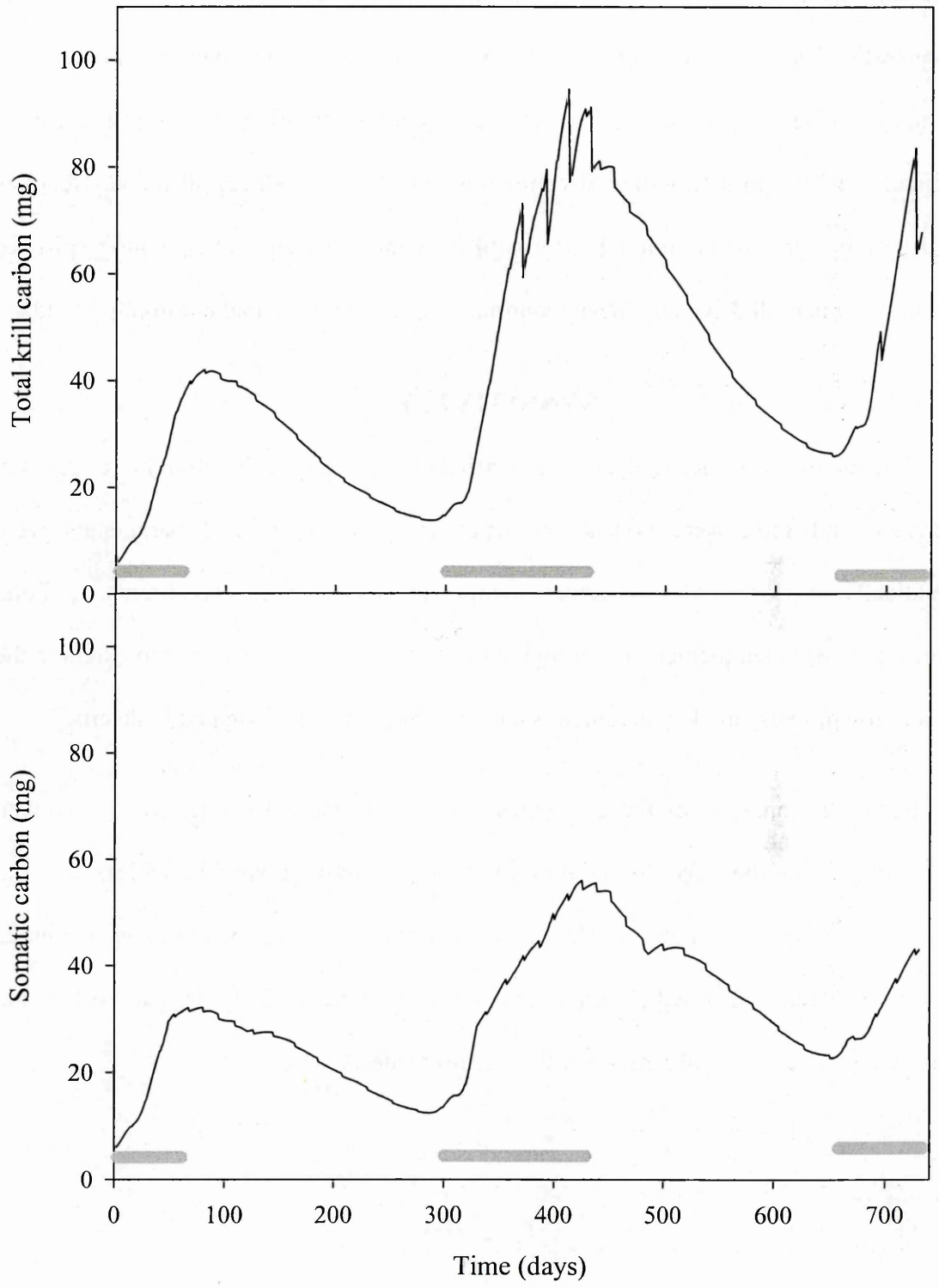

Figure 6.10. Results from the model run, as described above, for a krill initial length 22 mm, run for two years, with the annual cycle of temperature and chlorophyll *a* based on mean curves which repeat each year. Both total krill carbon and carbon stored in somatic tissue shown. Grey bars show period of phytoplankton bloom. Initial conditions were as described in Table 6.4.

Figure 6.10 shows the output from the model as parameterised above. Both total krill carbon and carbon in somatic tissue are shown. The model shows seasonal growth and degrowth. The large spikes in the total krill carbon figure show spawning events, with the smaller spikes visible in both figures representing moulting. This initial run is biologically realistic in that, unlike any previous model, it reproduces all the key features of krill biology; growth in summer, degrowth in winter, and several spawning episodes during the summer, all driven by mean environmental temperature and chlorophyll fields.

SENSITIVITY

A range of sensitivity analysis were carried out. Firstly the sensitivity to changes in temperature and food were considered separately, then individual parameters were systematically varied under constant temperature and food conditions. Food concentrations were supplemented, throughout the year, and only in winter to examine the effect on growth rates, and key parameters and variables were investigated in detail:

Sensitivity analysis of the energetics model as described above, was undertaken firstly by examining the effect of variation in the major environmental variables, food and temperature, on the model output. The model was run for a range of food concentrations (15–150 mg carbon m^{-3}) with temperature held constant at 1 °C (Figure 6.11) and sensitivity assessed by calculating short-term growth rates.

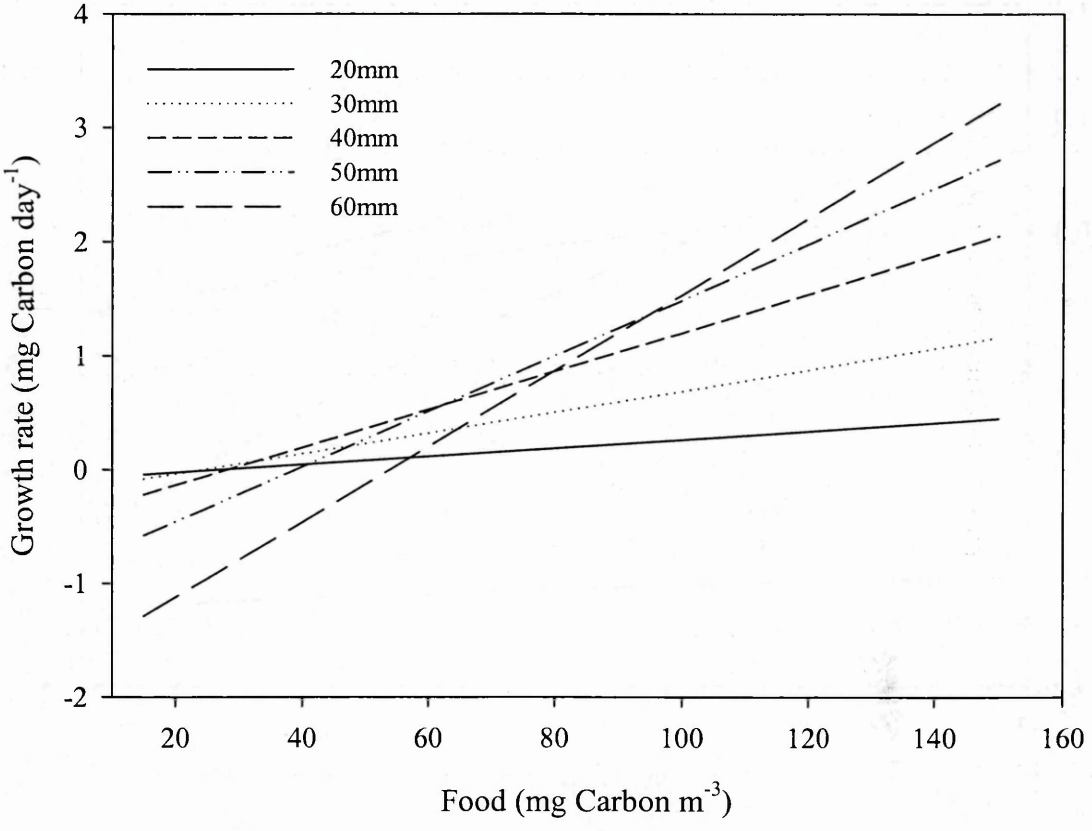

Figure 6.11. Growth rates of a range of sizes of krill in response to varying food concentrations. Temperature held constant at 1 °C. Growth rates recorded at day two of the model run.

The results show that growth is strongly influenced by food concentrations, which was to be expected. It was clear that variations in food availability had a much greater effect on the growth rates of larger krill, and that in times of low food concentrations, smaller krill were able to survive without displaying negative growth.

In a second analysis temperature was varied (-1.8 to 5 °C), whilst food was held constant at 75 mg carbon m^{-3} (Figure 6.12). It can be seen that food variation has a greater influence on krill growth than temperature, but that the size dependent effect of temperature is more dramatic for larger krill.

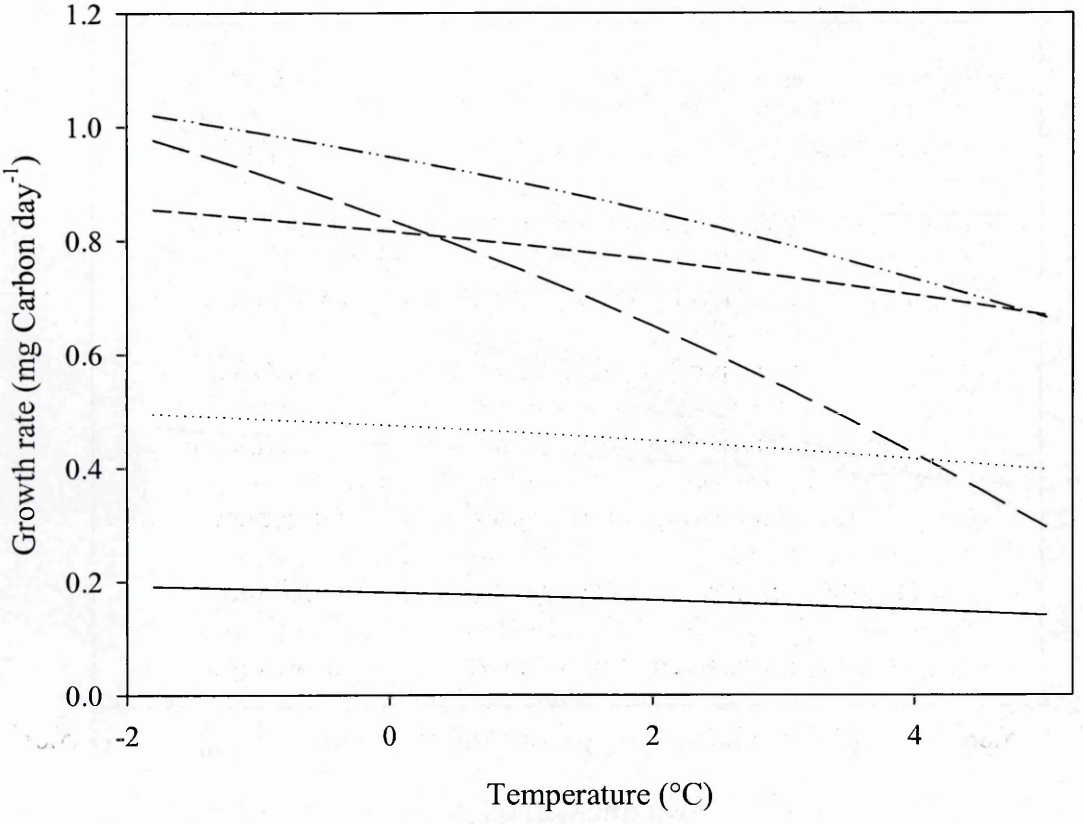

Figure 6.12. Growth rates of a range of sizes of krill in response to varying temperature. Food held constant at 75 mg carbon m-3. Growth rates recorded at day two of the model run. Legend as shown in Figure 6.11.

All the remaining parameters and variables were investigated for the sensitivity of growth rates in the model to a change in their values. For these model runs, temperature was held constant at 1 °C, and food at 75 mg carbon m^{-3}. Growth rates at day two and day 100 of the model run were recorded, and the number of spawning events, amount of carbon spawned and final carbon mass and krill length were recorded on day 100 of the model run, under these fixed conditions. One hundred days was considered long enough to allow spawning and moulting to occur and have an effect on the output of the model run. The results from this comparison are summarised in Appendix G, Table 2. In addition to this sensitivity analysis, the model was also run with temperature and food parameterised seasonally as previously described for conditions at South Georgia (Figure 6.7 and Figure 6.9), varying each parameter and variable in turn by ± 10%. Additionally, minimum and maximum values for the parameters available from the range of data in the literature were

tested in the same way, and the parameters ranked in terms of growth rate sensitivity (Table 6.5). The results from this form of sensitivity analysis, for a two year model run, are presented in Appendix G. More detailed discussion of the sensitivity of several parameters were then investigated separately, as discussed below.

Table 6.5. Rank of sensitivity of parameters in the model to a 10% increase and decrease in parameter value. Ranked from most sensitive (1, highest percentage change in carbon) to least (14).

	Total krill carbon			Somatic carbon		
Variable/ Parameter	Total	Summer	Winter	Total	Summer	Winter
Food	1	1	1	1	1	1
Filtration	1	1	1	1	1	1
Assimilation	1	1	1	1	1	1
Time feeding	2	2	2	2	2	2
Allocation to reproduction	3	3	4	3	3	4
Maintenance	4	4	3	4	4	3
Swimming	5	5	5	5	6	5
Start reproduction	6	8	6	6	5	6
Temperature	7	7	7	8	8	8
IMP	8	6	9	9	9	9
Spawn	9	9	7	13	13	11
Q_{10}	10	10	8	7	7	7
kg	11	11	11	11	10	12
Moult loss	12	12	10	10	11	10
Feeding cost	13	13	13	14	14	13
Repro decay	14	14	12	12	12	10

It is clear from the results of the sensitivity analysis, that the greatest effect comes from the variability of those parameters and variables associated with food intake. Indeed, the model shows the same rank of sensitivity to the effects of variations in food concentration, assimilation efficiency and filtration rate (Figure 6.13) when altered one at a time.

It can be seen that the krill display rapid growth in periods of the phytoplankton bloom, but during low phytoplankton concentrations, there is not enough food available to support growth, and rapid shrinkage is observed. Although krill have been shown to shrink in the laboratory and in the field in times of low food, the shrinkage rates observed here are

far greater than have been observed in the wild (see discussion below), and prevent the animals from gaining length beyond their second year (see Figure 6.14).

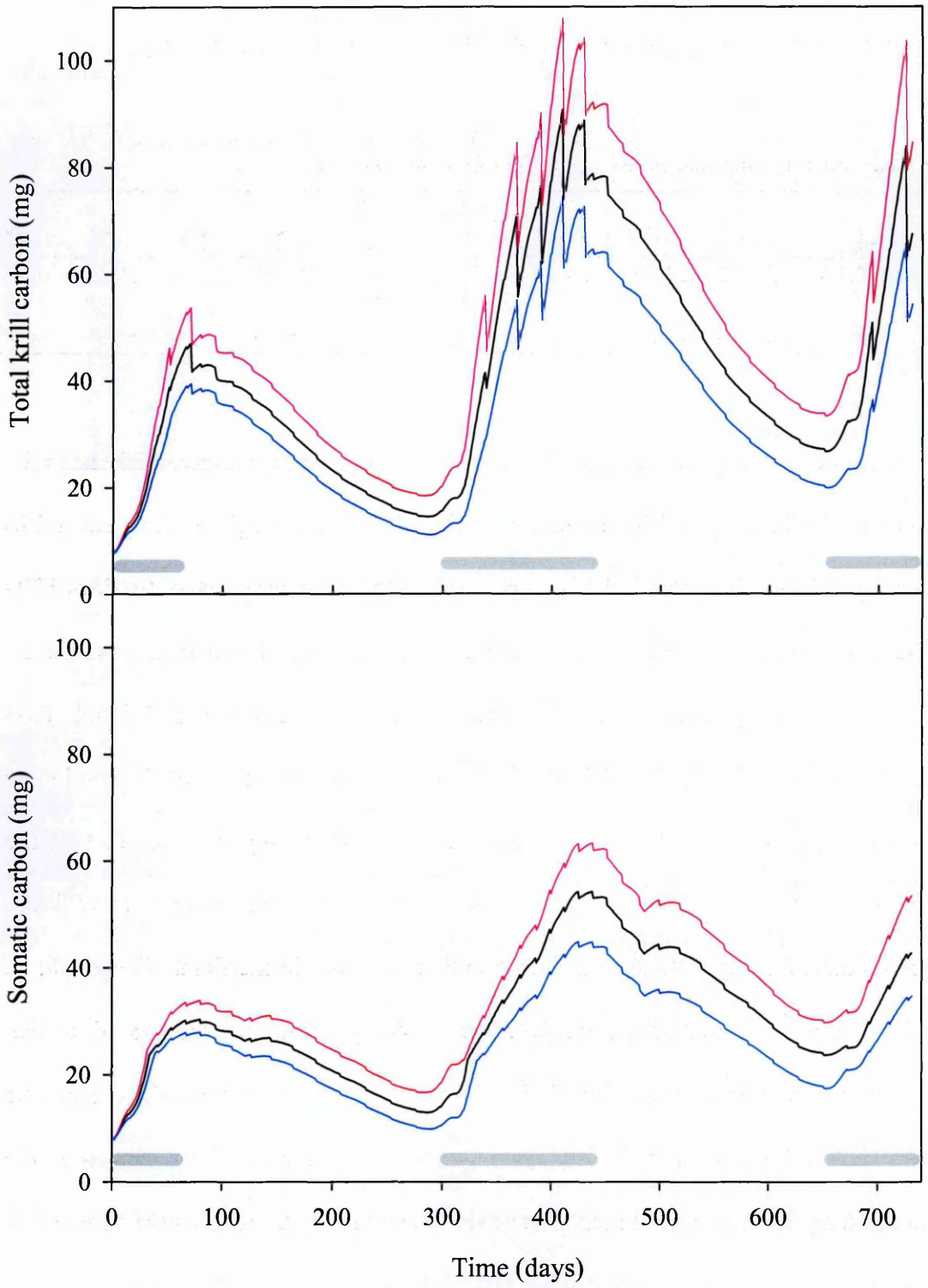

Figure 6.13. Change in total krill and somatic carbon content (black line) over time with a 10% increase (red line) or decrease (blue line) in food concentration (identical results obtained for variations in assimilation efficiency and filtration rate). Periods of phytoplankton bloom shown with grey bars.

Food concentrations which result in no net growth or shrinkage were investigated (where no moulting or spawning was assumed) and the results are presented in Table 6.6. The amount of food necessary to sustain krill, but not allow growth, increases with increasing temperature, and ranges between 0.44 mg Chl *a* m^{-3} (22 mg C m^{-3}) for a 22 mm krill at -1 °C to 1.21 mg Chl *a* m^{-3} (60.5 mg C m^{-3}) for a 60 mm krill at 3.5 °C.

Table 6.6. Food concentration required (mg C m^{-3}) to obtain a zero growth rate i.e. maintain the krill at the same size, under differing temperatures. No moulting or spawning assumed.

Length	Temperature (°C)									
(mm)	-1	-0.5	0	0.5	1	1.5	2	2.5	3	3.5
22	22.14	22.92	23.73	24.59	25.48	26.43	27.41	28.45	29.53	30.67
45	29.16	29.89	30.65	31.45	32.28	33.16	34.08	35.05	36.06	37.13
60	47.45	48.63	49.87	51.16	52.52	53.95	55.45	57.02	58.67	60.40

The effect of feeding the krill additional food on top of what was available from the phytoplankton was investigated by a) supplementing the food throughout the year and b) supplementing the food in the period of low phytoplankton concentrations only (days 120–280). The results of this are presented in Figure 6.14, for a model run starting with a 22 mm krill. Guided by the results presented in Table 6.6, three additional food concentrations were used: 25, 35 and 50 mg C d^{-1}. It should be noted that as the results are expressed in length, the reduction in length observed at spawning is an artefact of the carbon to length conversion, and would not be observed in field observations. In addition, the model simulates growth to a size greater than that observed in the field. This could be because the model can not realistically reproduce physiology at large sizes of krill, or that krill in the wild have the potential to reach these sizes, but they don't due to the constraints of food availability or other limiting factor. Additionally, as discussed before, the calculation of length from total krill carbon in the model is likely to be an overestimate.

As a response to the seasonal variations in food availability observed, it is likely that the krill may alter the time it spends feeding. Potentially, when low food

concentrations are encountered, the animal may spend less time feeding as a response. A scenario of reduced time feeding during the winter reduction in phytoplankton was hypothesised and tested in the model by reducing the time feeding during times of low food availability (days 120-280) to 50%, 25% and no feeding at all, however in all cases the benefit of a reduced cost of feeding was far less than that gained from the food consumed, even at low levels. It should be noted, however, that the real cost of feeding is not known for krill and the value in the model is only an estimation; should the cost of feeding be higher in reality, then krill would benefit metabolically from reduced periods of active feeding.

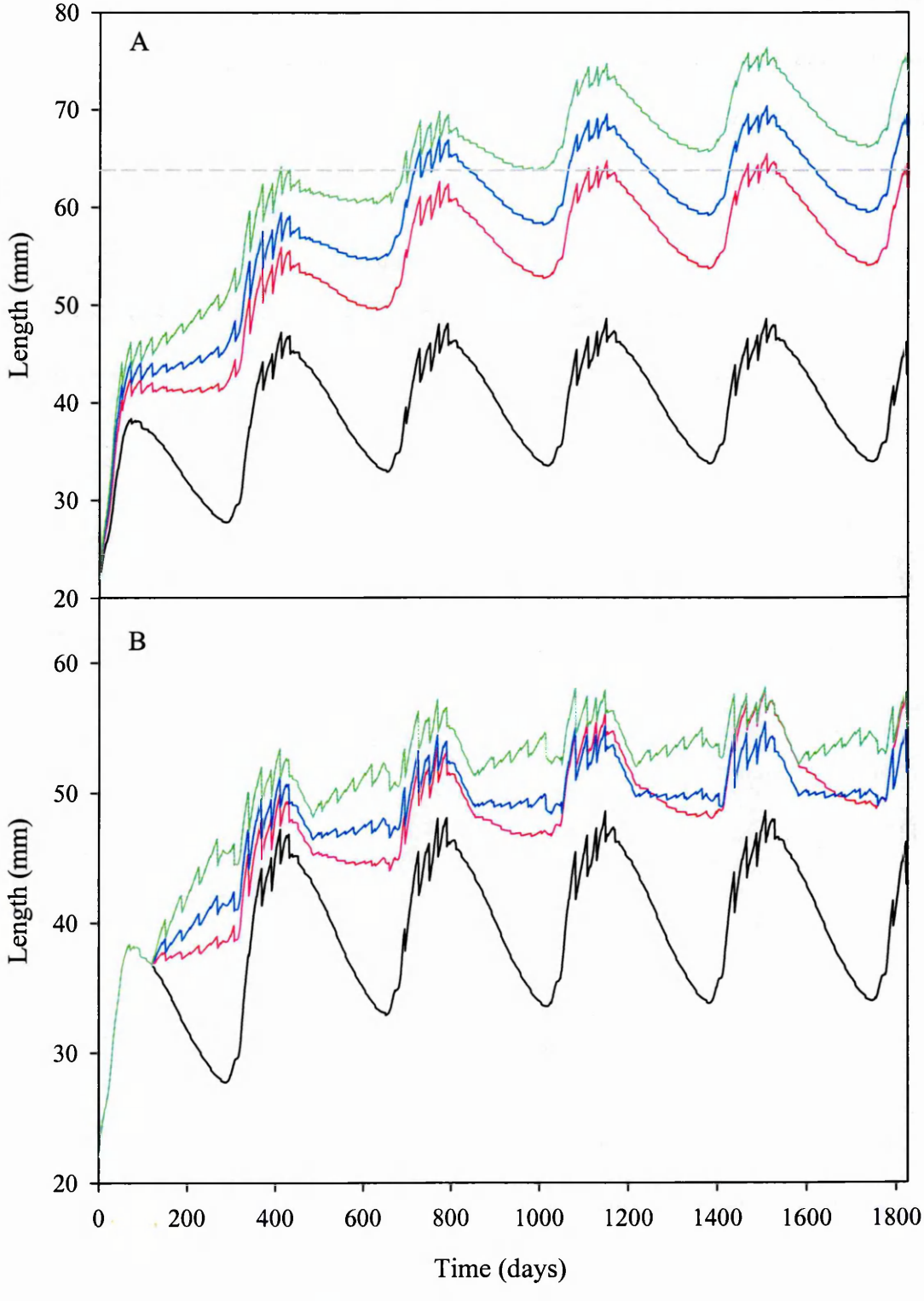

Figure 6.14. Effects of supplementing the phytoplankton food source available to the krill. Figure A shows a year round supplement of available food, figure B an increase only during winter. Black line shows original growth trajectory, red line increment of 25 mg C d^{-1}, blue line 30 mg C d^{-1} and green line 50 mg C d^{-1}. Dashed grey line represents 63 mm, considered approximate maximum length for krill under natural conditions.

The model is also sensitive to the costs associated with metabolism, which in this model are separated into the following components (maintenance, swimming and feeding costs and the energetic cost of growth). The total metabolic cost as calculated by this method was compared with those available in the published literature, as shown in Table 6.7.

Table 6.7. Comparison of model run metabolic rates with those from the literature. Environmental parameters for model metabolism results: temperature = 2.29 °C, food = 91.11 mg C. Q_{10} adjusted temperature represents adjusted metabolic rate for a 45 mm krill at 2.29 °C. † mean values calculated for juveniles and adults

Study	Season	Temperature (°C)	Metabolic cost (ml O_2 h^{-1}) 22 mm	45 mm	60 mm	Q_{10} adjusted 45 mm
Model run		2.29	0.0218	0.116	0.2475	0.116
Atkinson et al. (2002)	Autumn	-1	0.018†	0.039†	0.039†	0.034
Quetin & Ross (1991)	Summer	2	0.0298	0.107	0.246	0.110
	Winter	-1.5	0.00691	0.0313	0.0837	0.045
Clarke & Morris (1983)		-1	0.0065	0.0568	0.1356	0.078
Segawa et al. (1982)	Summer	0	0.0307	0.2385	0.5439	0.297
Quetin et al. (1994)	Summer	synthesis	0.0289	0.227	0.520	—
Hirche (1983)	Summer	1	0.0163	0.0827	0.238	0.094
Kawaguchi et al. (1986)	May	-1.5	0.00419	0.0572	0.1635	0.0824
	July	-1.5	0.00420	0.0354	0.0834	0.0510
	August	-1.5	0.00449	0.0521	0.1395	0.0751
	September	-1.5	0.0137	0.1624	0.4387	0.2339
Ikeda & Mitchell (1982)	Summer	-1.1	0.01031	0.0661 3	0.1521	0.0917

Temperature has three points of influence in the model: temperature scaling of metabolism (Q_{10}), the length of the intermoult period, and the spawning period (although this is determined by IMP), and so we would expect the model to be sensitive to the changes in temperature. It can be seen that the growth rate of krill decreases with increasing temperature (Figure 6.12), especially in larger krill. Since food intake is held constant in this analysis, these results indicate that growth efficiency declines with increasing temperature. This is caused by the greater maintenance costs at higher

temperatures. The temperature sensitivity is greatest in times of low food availability, as shown in Figure 6.15; in times of low food availability, krill do better in lower temperatures, due to a reduced metabolic demand and less frequent moulting.

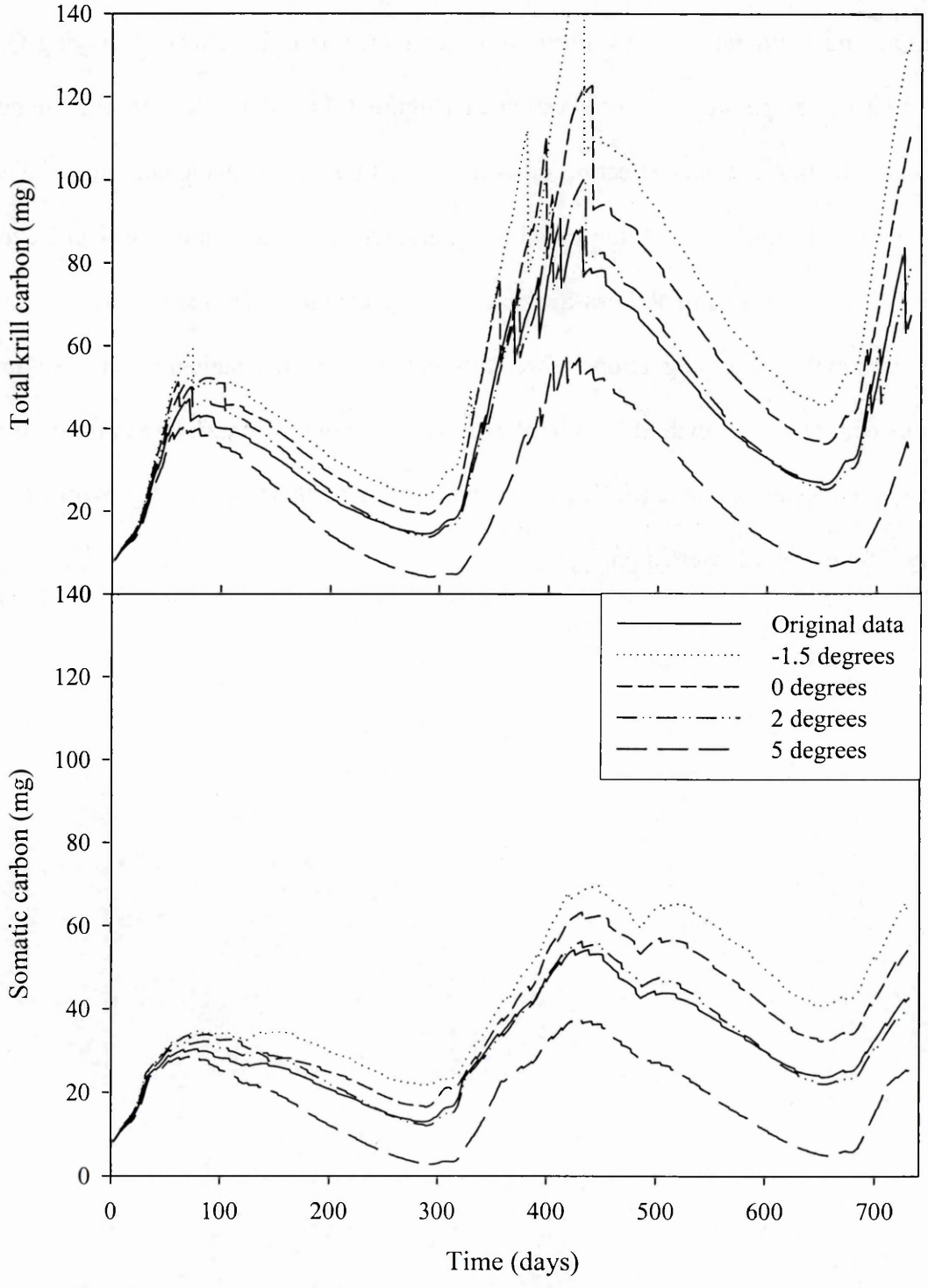

Figure 6.15. The effect of varying temperatures on the growth trajectory of krill, where temperature was held constant at the temperatures given in the legend, compared with the original temperature field as described in Figure 6.7.

As discussed in the model parameterisation section, accurate Q_{10} values for krill are uncertain. It has been suggested that they vary with both temperature and life history stage (Rakusa-Suszczewski & Opalinski, 1978). The sensitivity analysis where both food and temperature are held constant (Appendix G, Table 2) showed that an increase in Q_{10} value results in a decrease in growth rate and spawning events. However, as the interaction between Q_{10} and temperature is not linear (see Equation 6.10), the effect of varying Q_{10} over a range of temperatures was investigated (Figure 6.1). It is clear that at higher temperatures Q_{10} has a greater effect on growth rate, with a higher Q_{10} leading to reduced growth rates, most significantly at these high temperatures. This has some significance, for example if the conclusions of Rakusa-Suszczewski and Opalinski (1978) are correct, and Q_{10} decreases with increasing temperature, this could be a mechanism to combat the resulting effects of low growth at high temperatures with high Q_{10} values. Similarly, if it true that Q_{10} is higher in juveniles, then this would inhibit their potential growth rates, especially if temperatures were high.

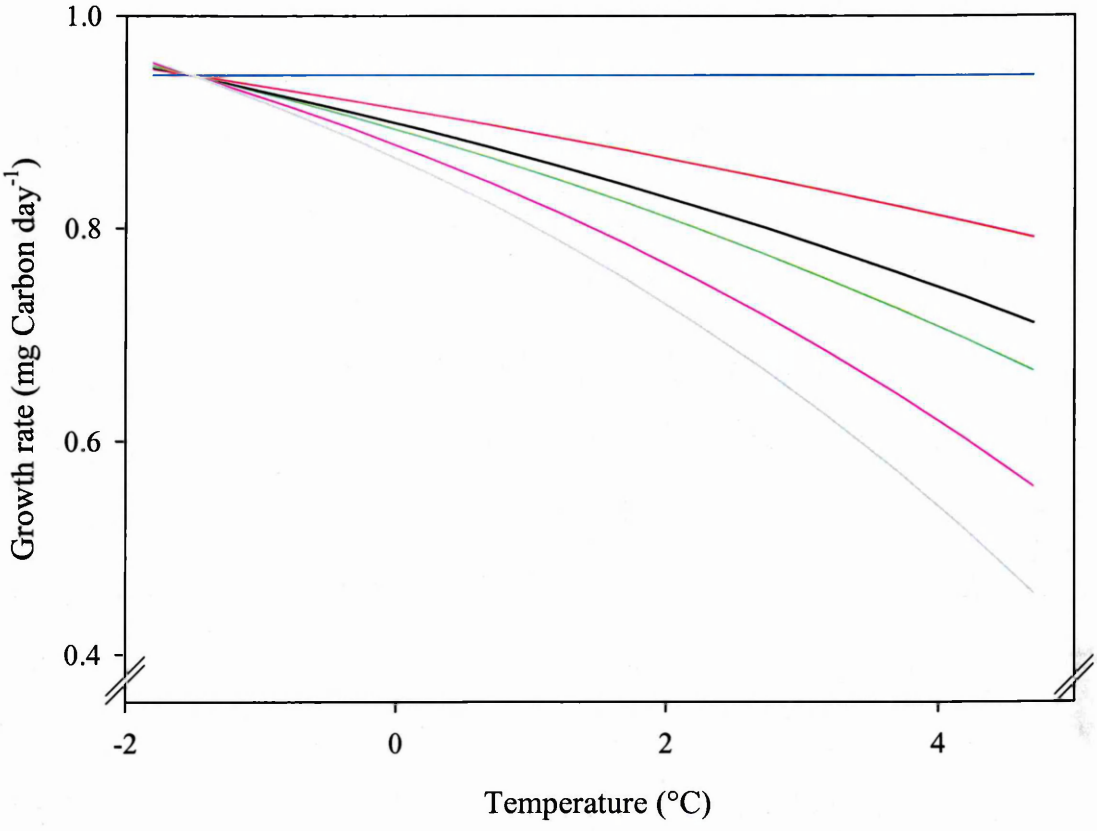

Figure 6.16. The effect of differing Q10 values on the growth rate of a 45 mm krill, over a range of temperatures. Growth rate taken at day 2, food = 75 mg C m^{-3}. Black line represent Q10 as parameterised in the model (2.62), blue line Q10 = 1, red Q10 = 2, green Q10 = 3, pink Q10 = 4 and grey Q10 = 5.

Similarly, there is an interaction between IMP and temperature, so considering the sensitivity of the model to IMP at a fixed temperature is also slightly misleading. Appendix G, Table 2 showed that a longer IMP leads to larger final lengths although growth rates are unaffected, as the frequency of spawning events is reduced. When evaluating the sensitivity of the model to several published IMP-temperature relationships, only slight differences are seen between the results for final length. The relationships of Tarling et al. (2006) showed an additional spawning event with the female specific relationships (FA and FS) compared to that for juveniles and the other studies.

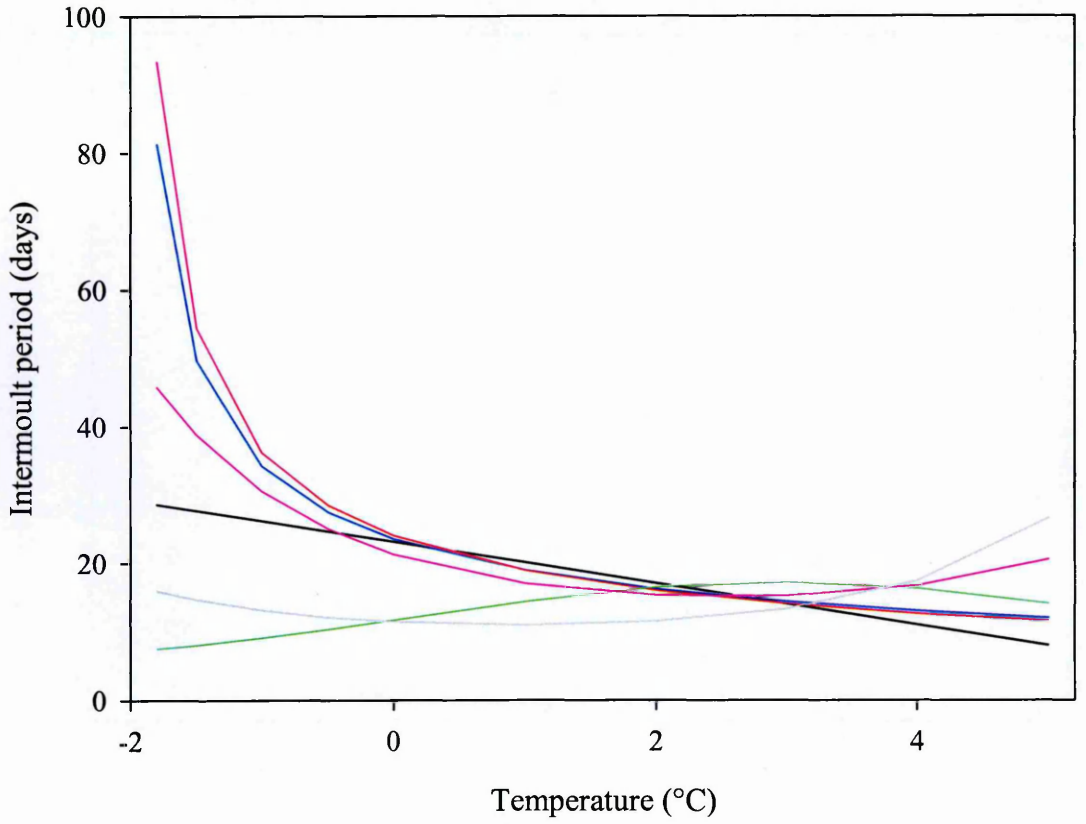

Figure 6.17. The relationship between temperature and intermoult period observed by a number of authors. Black line is the relationship of Buchholz (1991), blue Kawaguchi et al. (2006), red (Quetin et al. (1994), and the remaining relationships from Tarling et al. (2006) for krill of 40 mm, where the green line is for juvenile krill (J), pink for immature females (FS) and grey for mature females (FA).

When these relationships are compared (see Figure 6.17), we can see that the choice of IMP-temperature relationship is particularly significant at low temperatures for general relationships for all krill derived from published data (Quetin et al., 1994; Kawaguchi et al., 2006) and experimental results (Buchholz, 1991), the life history stage-specific relationships of (Tarling et al., 2006) show deviation from the other results over the full range of temperatures. (The relationships of Tarling et al. are size-dependant, so for comparison here, a fixed length of 40 mm has been used). However, when the sensitivity of the different relationships are compared in the model using the temperature series for South Georgia (see Figure 6.18, Appendix E), only the application of the relationship for mature female krill makes any significant difference to the krill, resulting in smaller sizes, most notably over winter, as moulting (and spawning) is more frequent.

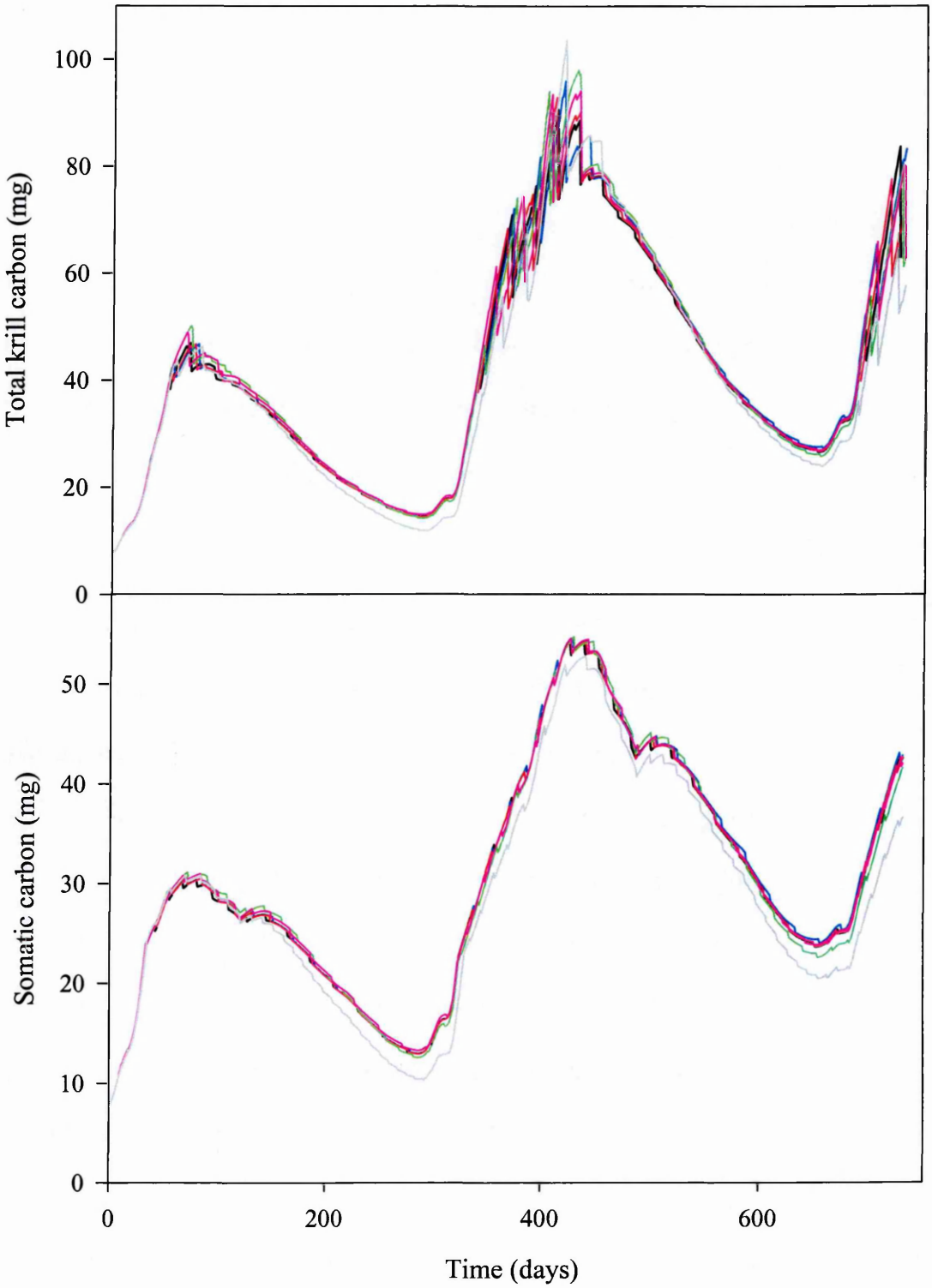

Figure 6.18. The effect on total krill carbon and somatic carbon of using the different IMP relationships in the model over a two year run. Black line is the relationship of Buchholz (1991), blue Kawaguchi et al. (2006), red (Quetin et al. (1994), and the remaining relationships from Tarling et al. (2006), where the green line is for juvenile krill (J), pink for immature females (FS) and grey for mature females (FA).

Reproduction is the other process that is sensitive in the model. It is intuitive that the parameter with greatest effect is '*allocation to reproduction*'. This parameter has had to be set arbitrarily, as no data are available to parameterise this physiological 'decision' of

how much of the krill's resources it 'chooses' to invest in reproduction. However, a review of the literature for a wide range of animal species by Koojiman, (2000) concludes that a value of 70% available energy partitioned to reproduction is the most appropriate general parameter. It is likely, that in addition to the size at which a krill starts allocating resources to reproductive development, and the amount of energy allocated to growth will both vary depending on the environmental conditions. It is not clear as to how much a krill 'makes a decision' to choose reproduction over growth and how much is related to environmental cues such as food availability, temperature and photoperiod. Figure 6.19 shows the effect on both total and somatic growth of varying the amount of energy allocated to reproduction.

It was originally thought that krill only spawned once a season (Mauchline, 1980a), but it is now recognised from field data that they have the potential to spawn up to seven batches of eggs per austral summer (Ross & Quetin, 1983; Ross & Quetin, 1986; Cuzin-Roudy, 1987a). In the original model run we can see animals spawning four to five times during the summer, which would tend to agree with these findings. We know that the frequency of spawning events is constrained by the moult cycle (Cuzin-Roudy, 2000), but the percentage of the developed ovary spawned at each spawning event is subject to variation. Average amounts of carbon spawned by the krill in the model are around 20% of total body carbon (ranging from 3 to 27%) which compares with values obtained compared by Nicol et al. (1995) who showed that krill lost an average of 34% dry mass (17% fresh mass) on spawning (Using a carbon content of ovary tissue determined by CHN analysis of 54.8%, 20% carbon mass is equivalent to 37% dry mass). The model uses, for tractability, a simplified relationship between oc1-oc2 and oc3-oc4 oocytes, which may influence details but not the overall pattern in the model output.

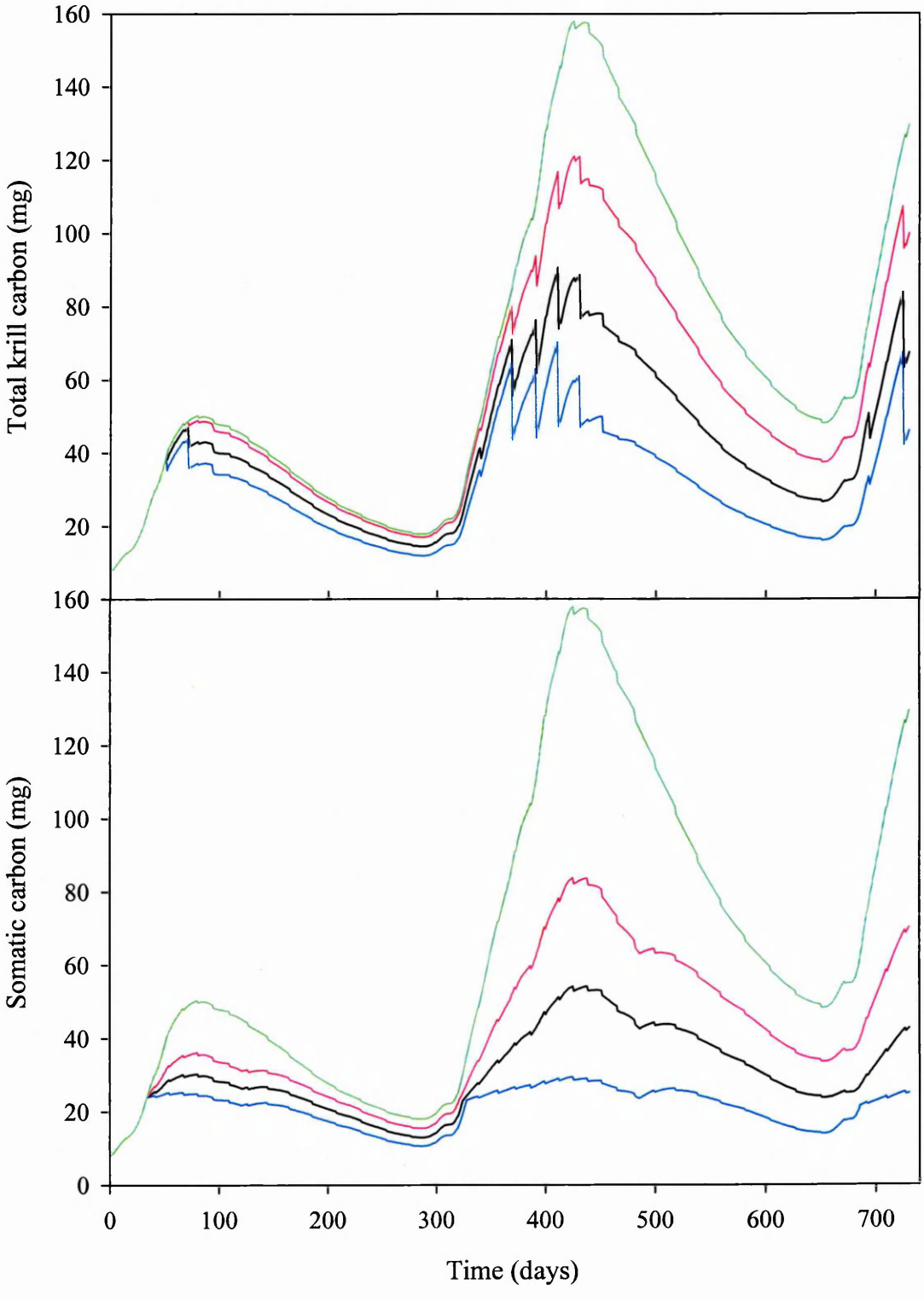

Figure 6.19. Effect of variability of the amount of energy allocated to reproduction. Black line original data (70% to reproduction), blue 90%, red 50% and green no energy allocated to reproductive growth.

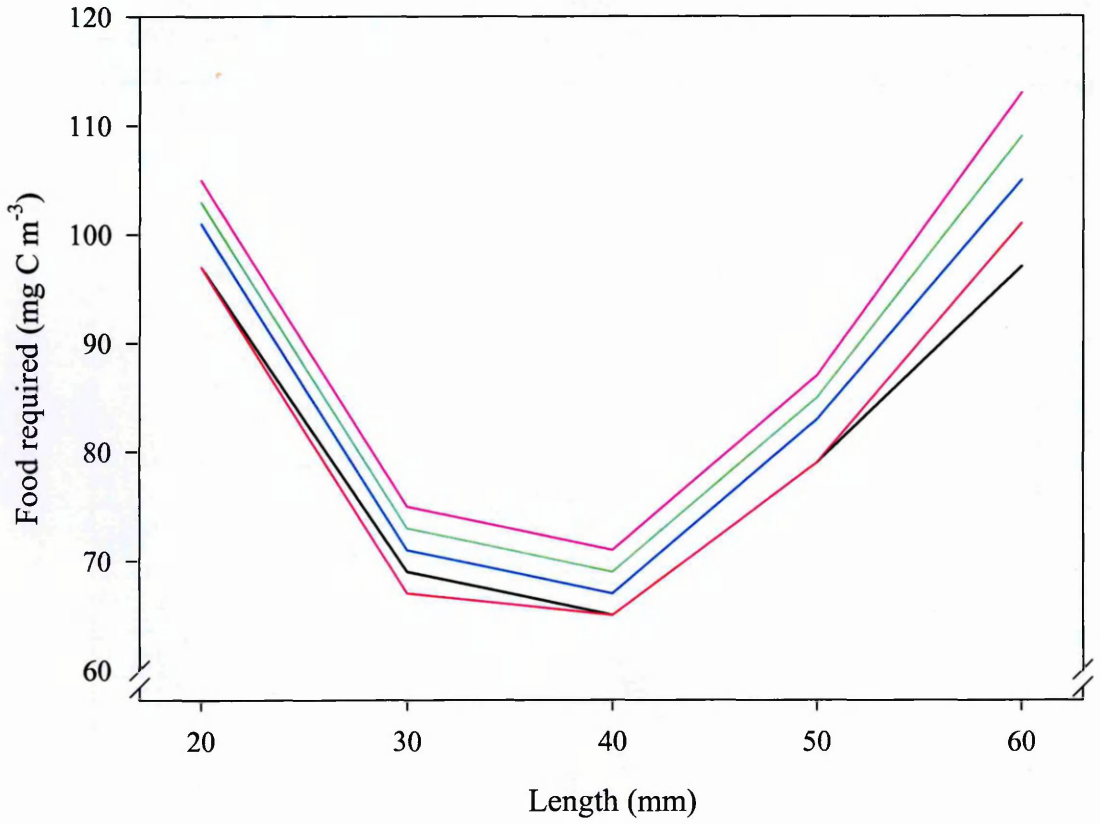

Figure 6.20. Concentration of food required per day to allow the production of 20 mg C mature ovary tissue in a range of lengths of krill, at varying temperatures. Black line represents temperature of -1.5 °C, red 0 °C, blue 1.5 °C, green 3 °C and pink 4.5 °C.

The output from the model reveals that there is a difference in the ability to produce mature ovarian tissue between krill of differing sizes. Figure 6.20 shows that krill of 30 and 40 mm require significantly less food to produce the same amount of ovarian tissue than juvenile, or more mature animals over a range of temperatures. For all krill ovary production is more efficient at colder temperatures.

The day on which the model is initiated also affects the growth trajectory, in the original run the first day on the model was taken to be January 1st, however timing of spawning and larval development rate affect the time at which the krill enter the furcillia feeding stage; taken as the size krill are initiated at in this model. Figure 6.21 shows the effect of varying the start date of the model (t=1). It can be seen that food availability at the point of model initiation, when juvenile krill begin to mature, significantly affects growth rate and reproductive success in the first year. Antarctic krill spawn during

summer (December to March) and larvae mature to furcilia stages by the autumn (Marr, 1962); the rate of development from hatching to last larval stage and the growth rate during this time and after affects time of year that krill enter this model.

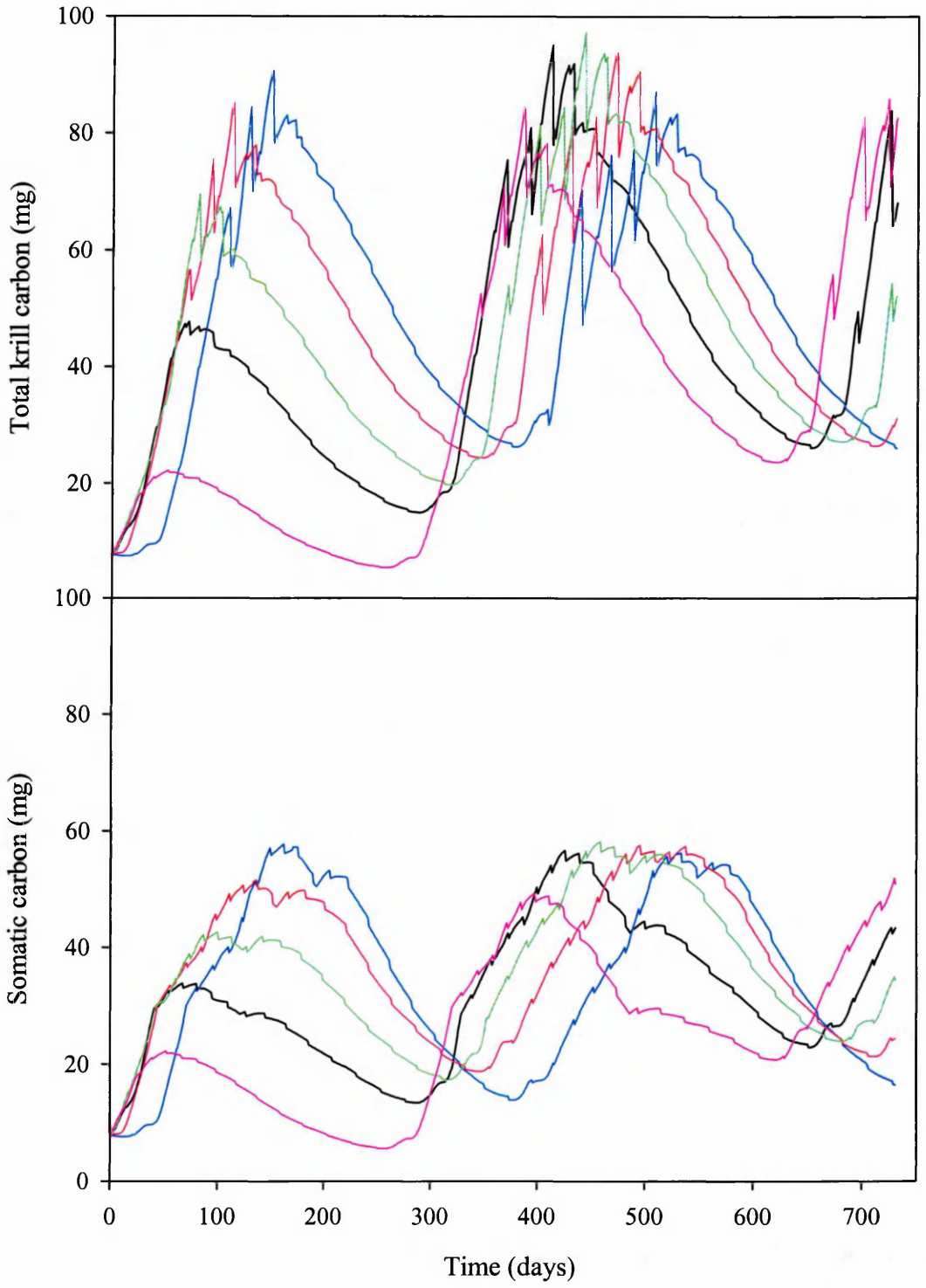

Figure 6.21. Effect of varying the date that krill enter the model simulation on total krill carbon and somatic carbon. Black line shows the original start date (t=1) of 1st January, blue line start date of 1st October, red line 1st November, green line 1st December and pink line 1st February.

Growth rates displayed by this model run, parameterised as described above, range from -0.06–0.45 mm d^{-1} at the extremes, with an average of about 0.2 mm d^{-1} for total krill length, and -0.05–0.5 mm d^{-1} (0.05 mm d^{-1} average) for somatic length.

DISCUSSION

Food

The models runs reproduce key features of the known biology of krill, which gives confidence that it is useful tool to explore the effects of environmental variability. The results of the initial model run show that levels of phytoplankton in the winter are not sufficient to support krill growth in the winter and periods of dramatic shrinkage are observed between May and September. This original configuration of the model assumes that the only source of food available to krill is phytoplankton as defined from SeaWiFS derived chlorophyll concentrations. Although Atkinson et al. (2006) showed that by far the best correlation with observed field growth rates was with SeaWiFS data, it is clear from the model results, that some additional feeding is needed to supplement the krill during the winter and times of low food. However, there is some evidence that krill feed on a range of food sources, including sea ice algae, microzooplankton food and detritus (e.g. O'Brien, 1987; Daly, 1990; Atkinson & Snÿder, 1997). It is also clear, from the provisional model results, that they must be utilising additional feeding sources in order to maintain year on year growth progressions that are routinely observed in the field, which is in agreement of the findings of Fach et al. (2002). Previous studies have also shown that krill are unable to meet their minimum carbon demands by consuming only phytoplankton (Antezana et al., 1982; Pakhomov et al., 1997; Perissinotto et al., 1997), suggesting the need for an omnivorous diet.

Previous studies have shown that krill feed on zooplankton in times of low phytoplankton abundance (Hopkins et al., 1993; Pakhomov et al., 1997), or engage in cannibalism (Mauchline, 1980a; Price et al., 1988). Evidence of Huntley et al. (1994)

suggests krill were metabolically and trophically active throughout the winter during periods of low phytoplankton (<0.05 μg chl l^{-1}) with no sea ice algae evident. It has been hypothesised that krill feed on copepods, which occur in high numbers around South Georgia, but are patchily distributed (Atkinson et al., 2001), with Clarke (1980) demonstrating copepod ingestion in the summer from krill lipid composition. Pakhomov et al. (1997) observed an average of ~53% micro- and mesozooplankton particles in krill stomachs from around South Georgia in February-March, a finding supported by other studies showing zooplanktonic remains in stomachs and faecal pellets (Marr, 1962; Tanoue & Hara, 1986). Kawaguchi and Takahashi (1996) showed Antarctic krill can feed on salps at a rate of 0.5 salp day^{-1} (for 30 mm salps) and suggested they may preferentially select them over other zooplankton. Pakhomov et al. (1997) suggest that herbivory may dominate only in spring and early summer when dense phytoplankton blooms occur , and that krill regularly switch from herbivory to omnivory during the austral summer to meet their energetic demands when phytoplankton concentrations are not sufficiently large to support its energy budget requirements. Huntley et al. (1994), Pakhomov et al. (1997) and references therein suggest the species is mainly carnivorous in the winter. Recent work by Schmidt et al. (in press) on the diet of krill from the Scotia Sea indicated the importance of heterotrophic food even in summer. They found diatoms to be most abundant in the stomachs, but with significant amounts of tintinnids, large dinoflagellates, and other armoured flagellates, although copepods were rare. Additionaly, krill have been observed feeding on suspended sediment (Clarke, pers. comm.) and benthic food sources (Daly, 1990).

So, how should these alternative food sources be quantified in order to better parameterise the model? Obtaining reliable estimates of zooplankton biomass can be tricky, as they are patchily distributed, although there has been plenty of work done on zooplankton biomass around South Georgia (Ward & Shreeve, 1999; Atkinson et al., 2001;

Ward et al., 2005; Ward et al., in press) and in the wider Southern Ocean environment (Boysen-Ennen et al., 1991; Hernandez-Leon et al., 1999; Hernandez-Leon et al., 2000; Calbet et al., 2005). We need to understand how *E. superba* feed on these zooplankton species to translate general zooplankton abundances to relative food availability; are they selectively predating certain species or sizes of zooplankton, or are they feeding indiscriminately on whatever they encounter? In addition we need to estimate prey consumption rate (equivalent to filtration rate) for this type of food. Huntley et al. (1994) exposed krill to copepods (dominated by *Oncaea* and *Oithona spp*), observing ingestion of between 44–356 copepods a day for 18–23 mm krill and 33–476 for krill of 37–42 mm (mean values of 181 copepods d^{-1} for the smaller krill and 243 for the larger animals). However, the ingestion rate was greatest in the first hour, declining rapidly thereafter, which may have indicated satiation or experimental stress.

Ward and Shreeve (1999) estimated total mesozooplankton abundances around South Georgia of 5.70 x 10^4 individuals m^{-2}, of which 2100 individuals m^{-2} were large copepods (*Rhincalanus gigas, Calanoides acutus* and *Calanus simillimus*) on-shelf and 2.35 x 10^5 and 8400 off-shelf. Copepods comprised 96–98% of the mesozooplankton biomass on-shelf, 81–86% off-shelf. Large copepods comprised 8–10% on-shelf and 40–50% off-shelf (although the on-shelf station was largely dominated by the small copepod *Drepanopus forcipatus*). In spring, copepod carbon mass and C : N ratio were low, which was to be expected in animals having overwintered. Residual biomass after removal of *Drepanopus forcipatus* was considerably lower than that observed in summer, principally due to a much reduced biomass of large copepods in the summer. At the oceanic station the small copepods (*Oithona spp, Oncaea spp* and *Metridia spp*) were numerically dominant, but biomass dominance was attributable to the large copepods. Carbon content ranged from 12-650 µg $individual^{-1}$ for *Rhincalanus gigas* CII-CVI females and 117-329 µg $individual^{-1}$ for CV and CVI female *Calanoides acutus*. Copepod abundances showed

variability between areas (WCB and an eastern sampling site close to transect TP059 (Figure 2.3)) and years (summer sampling 1995-1999) and ranged from 14,000 to 337,000 individuals m^{-2} (0–200 m) (Shreeve, 2002). Abundance was typically higher in the WCB than at the eastern site.

Worldwide, copepod carbon mass has been reported to range from c 28-63% of their dry mass (Båmstedt, 1986), with the range lying between 39-54% dry mass for Antarctic species (Conover & Huntley, 1991; Huntley & Nordhausen, 1995) for large copepod with older, lipid storing stages predominating. Shreeve (2002) showed a range in carbon contents of 6 µg individual^{-1} to 1037 µg in *Rhincalanus gigas* CI to CVI females and 4 µg to 463 in *Calanoides acutus* CII to CVI females. Carbon mass of *Oithona similis* in Castellani et al. (2005) ranged from 0.5 to 0.6 µg C total body content from the English Channel (typically 40% carbon), compared with Swadling et al. (1997), who found 0.78 µg C copepod^{-1} in *O. similis.*

Table 6.8. Amount of copepods needed (individuals day-1) to supplement the diet of krill by 25, 30 and 50 mg C d^{-1}.

		Supplement to phytoplankton concentrations		
Copepod species consumed	C mass µg individual copepod	25 mg C d^{-1}	30 mg C d^{-1}	50 mg C d^{-1}
Rhincalanus gigas	6	4167	5000	8333
	1037	24	29	48
Calanoides acutus	4	6250	7500	12500
	463	54	65	108
Oithona spp	0.5	50000	60000	100000
	0.78	32051	38462	64103

These data were used to estimate the number of copepods of different species commonly encountered around South Georgia a krill would have to consume in order to supplement its diet by the additional daily carbon values used in Figure 6.14, with the results presented in Table 6.8. These estimates are significantly higher than the only estimate of daily copepod ingestion given by Huntley et al. (1994) for *Oithona,* although it

is conceivable that krill could consume numbers of larger individuals of the bigger copepod species *Rhincalanus* and *Calanoides*.

It has been suggested in areas of sea ice, that ice algae may be a source of food for krill (Hamner et al., 1983), providing 10–100 times more Chl *a* than the water column below the ice (Daly, 1990 and references therein). Daly (1990) showed that detritus and protozooplankton are important components of the diet of larval krill overwintering under the ice, in addition to sea ice algae, and this may be similarly true for adults overwintering in the same conditions. Again, there is the need to consider how to best to incorporate this type of food into the model, as concentrations of sea ice algae are difficult to estimate (Arrigo et al., 1998b; Diekmann et al., 1998), and the mechanism for feeding on this type of algae is very different from active filtration of phytoplankton in the water column, because instead the krill actively graze the underside of the ice. Daly (1990) estimated that the Chl to carbon ratio in the ice was 37, although she hypothesised that in winter these ratios underestimate the amount of carbon available as the heterotrophic component of the biomass is greater than the autotrophic component, both in the water column and the ice. Gut contents of krill in this study showed larvae ingested heterotrophic organisms and detrital material in addition to phytoplankton or ice algae in the winter. In the case of this model, as it is parameterised here, krill from South Georgia would not experience sea ice being outside the area subject to seasonal sea ice (Zwally et al., 1983). However, this remains a potentially important source of food for animals at the Antarctic Peninsula or in the Scotia Sea, the hypothesised transport path of krill to South Georgia.

Perhaps an alternative measure of available carbon as a food resource would be particulate organic carbon (POC). Total POC includes all living (autotrophic and heterotrophic) and non living (detrital) particulate matter and when measures of POC are studied, they are 16–22 times higher than the autotrophic C : Chl ratios. During JR70, POC was measured, with median values for four groups of samples (defined by plankton cell

count) ranged from 163–352 mg m^{-2} for the top 0–50 m of the water column (Ward et al., 2005). Xiuren et al. (1996) recorded POC concentrations in Drakes passage of 88.9 μg l^{-1} and 106.4 μg l^{-1} in the Atlantic sector after filtration through a 280 μm mesh to remove larger zooplankton, whilst (Fabiano et al., 1993) recorded POC concentrations ranging from 101-498 μg l^{-1} from the Ross Sea after filtration through a 200 μm mesh. Krill have previously been shown to feed on detritus (Holm-Hansen & Huntley, 1984; Kawaguchi et al., 1986; Daly, 1990). Kawaguchi et al. (1986) showed krill feeding on detritus from the sea bed in coastal regions under the sea ice, but not this may not be possible in majority of areas where krill live pelagically and the continental shelf is very deep.

The relative contributions of herbivory, carnivory and detritivory to krill energetics have yet to be determined and the degree of selection exhibited by the krill to specific food types is not fully understood. Is their diet a passive representation of what is present in the water column or under ice surface or are they making active feeding choices dependant on food availability? I would suggest that, as the feeding basket of krill is so well developed for filtering phytoplankton, this is their feeding method of choice, and they only resort to alternative methods of feeding when phytoplankton levels are not sufficient to support growth. This would suggest a pattern of feeding as examined in Figure 6.14B, where we need to quantify the supplementary food in terms of type and carbon content. In addition, the respective filtration rates and assimilation efficiencies relating to these varied food resources are not well studied, but would need to be included in the model.

It is interesting to consider what level of food supports growth rates observed by previous studies (see Chapter 4). Table 6.9 presents growth rates derived from the model under different food and temperature conditions for a range of krill lengths. Table 6.9 highlights the differences in growth rate using the two different forms of growth rate estimation. Using total krill carbon is an overestimation of actual growth in length,

however using the somatic tissue to predict growth rate is sensitive to the amount of energy allocated to reproduction.

It can be seen from Table 6.9 that the model is able to reproduce observed growth rates successfully, under a range of conditions. Atkinson et al. (2006) observed a range of growth rates of 0.0134–0.318 mm day^{-1} for krill sampled during JR82, which the model is capable of predicting under comparable food and temperature conditions. Atkinson and colleagues concluded that the optimum conditions for maximal growth rates was small krill in cold water with plentiful food, a conclusion fully supported by the outputs from this model.

Although the shrinkage observed in the model is quite severe, it may not be entirely unrealistic; Ikeda and Dixon (1982) showed starved krill continue moulting and are able to reduce their body size, enabling them to survive 211 days at -1 °C without food by reducing their size without significant changes to chemical composition, that is tissue components were metabolised in direct proportion to their presence in the tissue. Quetin and Ross (1991) showed shrinkage rates between -0.16 and -2.03% body length at each moult, and Ikeda (1985b) observed that long-term food deprivation resulted in a relatively constant shrinkage rate of 0.033 mm d^{-1}, similar to that observed by Nicol et al. (1992b). This compares with a maximum shrinkage rate observed in the model of -0.06 mm d^{-1}, obtained with no supplementary feeding during the winter. Sensitivity analyses show that relatively minor adjustments to variables associated with food intake would bring model shrinkage into line with field measures. Alternatively the difference may indicate that maintenance respiration rates are slightly too high.

Table 6.9. Comparison of total krill carbon and somatic tissue growth rates on day two of the model run under a range of temperature and food conditions, for three sizes of krill.

Food mg C m^{-3}	Growth rate (mm day^{-1}) Somatic growth rate Temperature (°C)								Total carbon growth rate Temperature (°C)							
	-1.8	-1	0	1	2	3	4	5	-1.8	-1	0	1	2	3	4	5
22 mm																
25	0.02	0.01	0.01	-0.00	-0.01	-0.02	-0.03	-0.04	0.02	0.01	0.01	-0.00	-0.01	-0.02	-0.03	-0.04
50	0.11	0.10	0.10	0.09	0.08	0.08	0.07	0.06	0.11	0.10	0.10	0.09	0.09	0.08	0.07	0.06
75	0.20	0.19	0.19	0.18	0.18	0.17	0.16	0.15	0.20	0.20	0.19	0.19	0.18	0.17	0.16	0.15
100	0.29	0.28	0.28	0.27	0.27	0.26	0.25	0.24	0.29	0.29	0.28	0.28	0.27	0.26	0.25	0.24
125	0.38	0.37	0.37	0.36	0.35	0.35	0.34	0.33	0.38	0.38	0.37	0.37	0.36	0.35	0.34	0.34
150	0.46	0.46	0.46	0.45	0.44	0.44	0.43	0.42	0.47	0.47	0.46	0.46	0.45	0.44	0.43	0.43
45 mm																
25	-0.01	-0.01	-0.02	-0.03	-0.03	-0.04	-0.05	-0.05	-0.01	-0.01	-0.02	-0.02	-0.03	-0.04	-0.04	-0.05
50	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.07	0.07	0.06	0.06	0.05	0.04	0.04	0.03
75	0.05	0.05	0.05	0.04	0.04	0.04	0.04	0.04	0.15	0.15	0.14	0.14	0.13	0.12	0.12	0.11
100	0.07	0.07	0.07	0.07	0.07	0.07	0.06	0.06	0.23	0.22	0.22	0.21	0.21	0.20	0.20	0.19
125	0.10	0.10	0.10	0.10	0.09	0.09	0.09	0.09	0.31	0.30	0.30	0.29	0.29	0.28	0.27	0.27
150	0.13	0.12	0.12	0.12	0.12	0.12	0.11	0.11	0.38	0.38	0.38	0.37	0.37	0.36	0.35	0.35
60 mm																
25	-0.06	-0.06	-0.07	-0.08	-0.09	-0.10	-0.11	-0.12	-0.05	-0.06	-0.07	-0.07	-0.08	-0.09	-0.10	-0.11
50	0.004	0.002	0.00	-0.01	-0.02	-0.03	-0.04	-0.05	0.01	0.01	0.00	-0.01	-0.01	-0.02	-0.03	-0.04
75	0.03	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.08	0.07	0.07	0.06	0.05	0.04	0.03	0.02
100	0.05	0.05	0.04	0.04	0.04	0.04	0.03	0.03	0.14	0.14	0.13	0.12	0.12	0.11	0.10	0.09
125	0.07	0.07	0.07	0.06	0.06	0.06	0.05	0.05	0.21	0.20	0.19	0.19	0.18	0.17	0.16	0.15
150	0.09	0.09	0.09	0.08	0.08	0.08	0.08	0.07	0.27	0.27	0.26	0.25	0.24	0.24	0.23	0.22

Temperature

Although growth appears to be largely a function of food availability, the influence of temperature should not be underestimated. As shown in Figure 6.15, in periods of high food availability temperature variability has little effect on krill growth rates. However, when food is scarce, a significant benefit to the animals can be conferred by being in colder waters. The reduction in temperature has the result of lowering the metabolic rate and thereby reducing the daily energy demand, most markedly in larger krill. It could be hypothesised that krill would obtain as much of an energetic advantage from overwintering in sea ice from the reduction in temperature found there as from the added food available from ice algae. Indeed, it might be that the presence of food is merely a bonus to krill seeking to remain as cold as possible throughout the winter. Energetic benefits are a complex trade off between temperature and food, and under natural conditions, there is an interaction between the two variables; the highest food concentrations in the Southern Ocean are typically found at median temperatures, with higher and lower temperatures occurring when low food concentrations are observed. It is therefore unlikely that high food and high temperatures would occur simultaneously.

Overwintering

There are two schools of thought as to how krill overwinter. The first assumes that feeding ceases and that krill must reduce their metabolism, utilize stored energy or even shrink in size. Ikeda and Dixon (1982) showed that krill cannot survive the winter on stored lipid alone, instead they conclude that starvation and utilization of body protein was the only mechanism necessary to survive the winter. Quetin and Ross (1991) conclude that krill can survive the entire winter without feeding; they suggest that this is made possible by reduction in metabolic rate and that body shrinkage only accounts for a small fraction of the energy required for overwintering. They also suggest that in many areas, adult krill do not have the opportunity to feed on sea ice algae, so have no feeding, lowered metabolic

rates and negative or zero growth rates. The key point here, however, is that metabolic rate must decrease if there is no feeding or growth.

However, the second school of thought is that krill do feed and grow in the winter. Winter feeding has been observed; for example Lancraft et al. (1991) showed a variety of phytoplankton in the guts of krill in the Scotia Sea in winter. Morris and Priddle (1984) also observed krill feeding on phytoplankton in the water column in winter. There are field observations which suggest krill continue to increase in length in winter, which suggests krill are feeding on other sources of food (as discussed above) since phytoplankton concentrations at this time are inadequate to support growth, (Hofmann & Lascara, 2000; Fach et al., 2002). However, McClatchie (1988) tracked krill length through winter and observed a decrease in size despite observed feeding, so it is likely, in this case that the food resources were insufficient to prevent shrinkage. Price et al. (1988) suggested feeding on zooplankton was insufficient to meet metabolic requirements. Copepods have been consistently found in the guts of krill in summer (Hopkins 1985), autumn (Hopkins and Torres 1989) and winter (Lancraft 1991). Huntley et al. (1994) showed no significant differenced in C : N ratios in faecal pellets between summer and winter indicating that the krill were feeding on similar food. It seems that the simple picture is that krill feed if they can, and this may or may not prevent shrinkage, but do not feed if there is no suitable food (of any type) available.

Success of krill at South Georgia

South Georgia is warm, so model results indicate that in absence of winter feeding krill will shrink. It is possible that there is sufficient alternative food available in the form of copepods and POC, but it remains uncertain as to whether the krill are able to exploit it efficiently enough to avoid shrinkage. The presence of krill in the waters surrounding South Georgia is mainly dependent on passive transport of animals into the area via surface currents (Thorpe et al., submitted). The Scotia/Weddell confluence has one of the highest

concentration of krill larvae in the southern ocean (Miller & Hampton, 1989) and is considered to be a primary nursery for krill (Marr 1969; Brinton and Townsend 1984); krill are both spawned in this area and transported in from the major spawning ground north and west of the Peninsula. Krill maintained at South Georgia have to contend with high water temperatures and a seasonal phytoplankton peak, which the model has shown to be sub-optimal conditions for krill growth, yet reproduction is clearly possible, although the idea that South Georgia has a self-sustaining population is widely debated. It may be, that while krill can successfully spawn several times a season, the environmental conditions may not be suitable for embryo development (Hofmann & Hüsrevoglu, 2003).

Previous energy budgets

Previous attempts to develop an energy budget for krill have been undertaken by Clarke and Morris (1983), who calculated an approximate budget for adult krill in the summer with the main aim of highlighting where data important to the development of a functioning energy budget were lacking. They concluded that a male krill, in the summer, requires 5% body mass a day and 6% or more for females. They compared this value to that obtained by Chekunova and Rynkova (1974), also 5% body mass per day, calculated from oxygen consumption and food intakes. Miller et al. (1989) synthesised various aspects of the energy budget of krill using different parameter estimates from Clarke (1983), but produced a very similar estimate of 4.1% body mass carbon individual day^{-1} for an area of the Indian Ocean. However, when Clarke et al. (1988) made experimental measurements, he recorded values of up to 20% in mature male krill (pers. comm.). Results from this model show a maximum requirement of 5% a day, with a typical average requirement during summer of around 3%, indicating ecologically meaningful results at intake rates well below those physiologically possible.

Aspects of the ecology of krill not included in the model

There are some well known aspects of krill ecology that have not been considered in this formulation of an energetics model. As the model considers physiological requirements and decisions, key aspects of krill behaviour are not included, notably, swarming and diurnal migration. Krill migrate to the surface at night to feed, whilst spending the day at depth, although Tarling and Johnson (2006) have recently suggested that krill feed at the surface, sink into deeper water when full and then swim to the surface again once digestion is completed, repeating this pattern up to three times in a night. As temperature and food availability vary with depth (see Appendix H), temperature and food concentrations are therefore not constant throughout the day. As krill migrate to depth, where waters are cooler when they are not feeding, their metabolic rates (and hence energetic losses) are reduced, conferring an additional benefit to migration. Similarly, krill may not be feeding 100% of the time – we can either assume that they are speculatively feeding all day in case food is encountered even at depth (even if at potentially reduced concentrations), or that they are only actively feeding during the night. For the purposes of this model it is assumed that the effects of diurnal migration are averaged out over a day, and as the model runs over a time step of one day, the assumption is made that the temperature and food conditions experienced are an average of those encountered at the surface and at depth.

The benefits of being in a swarm have not been included in the model so far, as it was unclear precisely how this could be done. However, it could be hypothesised that the krill receive a benefit from swarming in terms of energetic cost (reduced cost of swimming due to the reduced effect of hydrodynamic considerations, and potentially, protection from predation to reduce the amount of swimming needed in attempting to avoid predators). Disadvantages of swarming in low food concentrations are that food might become depleted quickly with less available for any individual krill, although in high densities of

phytoplankton this is likely to be a negligible affect. In addition, it is possible that oxygen could be reduced and ammonia elevated in dense swarms, though we do not know whether either of these would be at levels to elicit specific negative physiological effects. Similarly, we do not have physiological measurements of krill in a swarm; nearly all laboratory measurements are carried out on individuals, so it might be unsafe to assume that these rates would be the same in a swarming animal. It is likely that any net effect of swarming would be a benefit, so by not including it in the model, we can assume the energetic requirements described by this approach are some what of an overestimate.

As discussed previously in Chapter 4, krill do not grow continuously, rather they grow in steps, associated with moulting. However in the model, growth is assumed to be a continuous process, with growth rates during an intermoult period adding to a total increase in length that would be observed at the point of moulting. A useful development of the model would be to allow the growth to take effect only at the moult, especially when length is being used as a reference in physiological rates.

Problems and lack of data

The model has highlighted the need for more targeted physiological investigation on several aspects of krill energetics, notably swimming costs, metabolic costs of growth and feeding, Q_{10}, more detailed diet information and functional responses to feeding on different food types and the amount of resources krill allocate to reproduction under range of conditions.

Although we might have reliable methods of determining physiological relationships, we still do not have a comprehensive understanding of the effects of size and, more especially, sexual maturity, on these relationships. Adopting this strategy in physiological studies is not without difficultly, but the effects of life history stage are not to be underestimated. For example, we might hypothesise that egg production in mature

females has a significant effect on metabolism, or more particularly on swimming costs (as greatly increased body size will significantly increase drag). Physiological rates in mature males may be influenced by the energetic cost of reproduction; production of testis and metabolic cost of reproductive behaviour (see discussion in Chapter 3).

Advantages of this model and recommendations for future use

Refinement of the Hofmann and Lascara (2000) model by restructuring the parameterisation of respiration, and addition of moulting and spawning has produced an ecologically realistic simulation of growth and reproduction in female krill. This marks a significant development in our understanding of krill energetics. Sensitivity analysis has indicated that, as might be expected, krill energetics are affected most strongly by food and temperature (and their interaction).

Advantages of this structure of energy budget are that it allows for incorporation of better data when it becomes available and should be revisited regularly as the breadth of knowledge and experimental techniques improve. The model is also highly flexible in that factors for diurnal migration and swimming speed etc. can easily be added. As it is a discrete time implementation, the incorporation of this model into coupled ocean transport models and large scale food web models would be possible, to enable more accurate predictions of ecosystem function.

The current model could be improved by developing a sexual maturity stage dependant model, where morphometrics and physiological rates are linked to sexual maturity stage enabling more accurate predictions of energetic responses. Also the model should be developed for male krill, as they have very different reproductive energetics and may potentially display quite different growth responses, however a better understanding of male reproductive energetics is needed, although estimates could be made.

Chapter 7 Discussion

MAIN FINDINGS OF THIS STUDY

In this thesis I investigate the chemical and elemental composition and physiology of krill in relation to life history and environmental factors. Variability in proximate and elemental composition has been observed as well as in measured and predicted growth rates. We have observed variability in the elemental and biochemical composition of krill collected in the field between life history stages, season and sampling location; in measured field growth rates; and in variability of the responses of krill life history in model simulations, such as growth and reproductive capacity. I hypothesise that these variations in chemical composition reflect the observed physiological responses in krill life history that are influenced by environmental conditions.

The principle findings reported in this thesis are:

The chemical composition of krill was observed to vary between individuals and with sex, maturity and moult stage. Neither length nor mass alone can be used as a predictor of proximate composition, as these relationships are complicated by the effects of sexual maturity. Instead, this study highlights the importance of considering the variability in proximate and elemental composition with sexual maturity stages when considering changes in body chemistry in relation to environmental factors. Complex interactions of sex and maturity, season and environmental influences, and growth rate and moult cycle exist, but the low numbers of animals and lack of necessary replication, meant that this study was unable to define all of these interactions. However, it is clear that physiological changes relating to progression through maturity stages and physiological and behavioural responses to the environment in which the krill is living are reflected in its chemical composition. Trends in the chemical make up of krill and the variation in this make up

with maturity stage indicate that with more work, detailed investigations of aspects of chemical composition can provide insights into the physiology and ecology of organisms.

I have shown that changes in chemical composition between individual krill are linked to their physiology, so the question arises can chemical composition be used as a proxy for physiological process or rates? I have attempted to investigate the relationship between chemical composition and one aspect of life history; growth rate.

It was hoped to demonstrate a predictive relationship, between elemental composition and observed growth which would aid our studies of the interactions between growth and environmental variability. It was shown that growth rates in krill were highly variable, and displayed a range of growth responses from negative growth to rapid growth in summer, all in a region of generally high productivity. Although a theoretically predicted relationship between individual short-term growth rate and nitrogen and phosphorus stoichiometry was not supported by the observations of this study, it was demonstrated that mean data for *E. superba* from this study fitted the general interspecific relationship between elemental stoichiometry and growth rate previously described for freshwater zooplankton. This indicates that the hypothesised relationship between N : P stoichiometry and growth does obtain results over longer timescales. Despite the short-term relationship not holding for individual krill, this work suggests that there remains merit in the idea of searching for biochemical proxies for growth and other physiological responses, which would enable us to better investigate responses to environmental variability which are essential to the development of successful, predictive energy budget models.

A complete understanding of species ecology and ecosystem function requires knowledge of the relationships between biochemical composition of organisms and their environment. Chapter 5 demonstrates how it is possible to obtain the resolution and

insight gained from a complete understanding of the proximate composition of an organism from analysing only aspects of its elemental composition, a much quicker approach which allows for increased replication and smaller sample size. By applying modified stoichiometric theory to krill it was possible to construct a realistic chemical composition for all life history stages from elemental composition data which reflects that which was directly measured in Chapter 3. The ability to move from a reliably known elemental composition to an estimated proximate composition has many applications, not least in the field of energy budget modelling.

The final chapter describes the development of an energy budget model for Antarctic krill, which comprises a novel representation of the energetic costs of growth. This model was used to explore key aspects of the life history of krill, such as growth, maturity and reproductive success which were investigated under a range of environmental conditions, and a range of over-wintering strategies were examined. Investigations using this model allowed us to predict the implications of changes in environmental variability on the ability of krill to survive and reproduce. It was evident that food availability and temperature have a highly significant role in determining the life history of krill. Most notably, in times of low food, krill (especially adults) are better able to survive in areas of low temperature due to decreased metabolic costs.

IMPLICATIONS FOR THE SOUTHERN OCEAN (SOUTH GEORGIA) ECOSYSTEM

Krill are fundamental to the Southern Ocean ecosystem, as discussed in the introduction, and fluctuations in the krill population can have wide reaching implications.

Krill like it cold – the effect of temperature variability

High latitudes are the fastest warming regions on the planet (Vaughan et al., 2003), and the climate of the Western Antarctic Peninsula is the most rapidly changing in the Southern Hemisphere, with atmospheric temperatures rising nearly 3°C since 1951

(Meredith & King, 2005). The Southern Ocean is also showing clear signs of warming (Gille, 2002), with Meredith and King (2005) showing major oceanographic changes have occurred in one of the key nursery and spawning areas for krill, the waters adjacent to the Western Antarctic peninsula, during the second half of the twentieth century with summer surface water temperatures rising more than a degree. I have shown in Chapter 6 that krill are sensitive to fluctuations in temperature and Atkinson et al. (2004) demonstrate that krill show a significant dependence on the physical environment. The model simulations show that an increase in water temperature could have a significant impact on the physiology of krill, notably through the interactions with food, so any increase in sea temperatures could have a serious impact on krill energetics at times of low food and hence on the functioning of the ecosystem. Indeed, this ecosystem impact has been detected in higher predators by Forcada et al. (2005), who showed that sea surface temperatures had an effect on the breeding success of Antarctic fur seals, *Arctocephalus gazella.* The diet of fur seals is largely based on krill (Reid & Arnould, 1996) and their population dynamics seems to be controlled by bottom-up processes driven by the availability of krill (Forcada et al., 2005). Forcada et al. (2005) showed unusually warm summers were followed by significant breeding failures in fur seal populations at Bird Island, South Georgia. They concluded that the persistence of high sea surface temperatures altered the local environment through physical and biological interactions. These processes subsequently affected Antarctic fur seal females over long time scales, over which reductions in pup production could be observed and predicted. The authors hypothesised that this linkage between climate variability and pup production is related to changes in the distribution and abundance of Antarctic krill, the main prey item of the fur seals (Reid & Arnould, 1996; Murphy et al., 1998). In addition Fedulov et al. (1996) analysed catch data from the krill fishery around South Georgia, finding krill abundance to be greatest during periods of cooler oceanographic conditions. Shreeve (2002) demonstrated that the krill biomass recorded

around South Georgia over a number of years decreases with increasing water temperature in the top 60 m. These bottom-up effects are driven principally by changes in krill distribution and abundance. They would be exacerbated by decreases in krill condition or size (by reducing energy available to predators).

Krill abundance at South Georgia is variable, with pulses of recruitment (Murphy & Reid, 2001), and with the changes in recruitment, retention and increase in biomass thought to be related to oceanographic variation following meso- or large-scale movements of the southern Antarctic Circumpolar Current front (Thorpe et al., 2002). Trathan et al. (2003) suggested that high krill biomass at South Georgia only occurred when oceanographic conditions were colder; with oceanographic current variability providing the explanation. They suggested that temperature variability in the southern portion of the ACC influenced the biomass of krill reaching South Georgia. However, the physiological implications of temperature variability on the energy budget of krill, and the observed decrease in growth rate of krill with increasing temperature, as shown in the model, should not be over-looked. It is highly likely that this physiological effect of temperature on animals is contributing to the reduction in biomass, previously only attributed to oceanographic shift in current altering the supply of krill. Krill that are transported into areas of warmer waters, especially at time of low food, may display low or negative growth rates, or may even fail to survive the winter.

We can see that the effects of climate variability affect krill and these effects cascade through the food web to impact on the success of higher predators. It is possible that the temperature sensitivity demonstrated by krill in the model developed in this study could be an underlying factor to the poor success of krill in years with a high temperature anomaly, especially if high temperatures occurred through into the winter or were coupled with low food availability. Forcada et al. (2006) suggest the same relationship exists with other higher predators that feed on krill (in this case penguins), highlighting the

interdependences in the Southern Ocean food web and the critical role which krill plays within this system. We must continue to focus our efforts on implementing and improving krill energetics models, especially with a focus on temperature dependence, in order to improve our results using krill models in a predicative capacity.

Krill have traditionally thought to have befitted from sea ice in the winter as an additional source of food, but if they can also confer an energetic advantage by remaining in an area with low temperatures, they may benefit as much from the effects of the reduced temperature as the additional food supply. Indeed, it could be suggested that the ice algae availability is an additional benefit, on top of that conferred by of krill encountering areas of low temperature in which to overwinter. During transport across the Southern Ocean towards South Georgia, krill must encounter areas of low temperature in order to survive (Thorpe et al., submitted) and that these areas of low temperatures and sea ice (with associated food) are important in dictating krill survival and condition over winter through the mechanisms demonstrated in the energetics model.

LIMITATIONS OF THIS STUDY

The principle limitation of this study, in terms of elucidating patterns in the chemical composition of krill with respect to sex, maturity and environment, stems from the lack of sufficient numbers of krill for rigorous statistical testing. Ideally krill would have been collected in sufficient numbers at each sampling site to allow for a rigorous experimental design to be implemented; in reality this was not possible. The aim was to collect enough krill of all sex and maturity stages at a range of sizes from each sampling location, so that there was replication of each stage at each site and between sites and between years. This would allow for some of the variability attributed to environmental conditions or year effects to be tested for. However, as is the nature of field sampling of wild populations, not all maturity stages were present at each sampling location, indeed some swarms were dominated by krill of a certain size range or balance of sexual maturity

stages. The other problem with the numbers of animals required for such an experimental design, is that the time taken to do the chemical analyses would have been prohibitively long. However the chemical work reported here shows that the more rapid elemental analysis can tell us as much as the more time consuming proximate analysis. So, although a better selection of animals would have improved the quality of the results from the chemical analyses, this was not achievable practically.

DIRECTIONS FOR FURTHER RESEARCH

An original aim of the study was to further develop the energetics model to run not only tracking carbon, but also nitrogen, phosphorus and hydrogen. This would provide the link between the chemical changes in life history stages observed and the physiological changes with life-history that are implicit in the model. With the detailed stoichiometric calculations developed by this study it is possible to track the partitioning of the elemental resources in the krill into protein, lipid, carbohydrate (principally) at various stages in a krill's life history. This study focussed on model development and sensitivity analysis together with the development of suitable chemical methods for krill and the detailed description of the chemical composition of krill life history stages. It provides the basis for this advancement in energetic modelling to be undertaken, to shed some light on those aspects of the energetics of krill not yet understood, and to better parameterise the metabolic costs of life for krill. Aspects of krill ecology such as the discussion as to the degree of lipid storage and utilisation as an energetic reserve for over-wintering (Hagen et al., 2001) could be investigated in this context. As a next step, this important next step in energetics modelling is achievable, combining the aspects of the variation in chemical composition with life history stage, the stoichiometric calculations and the structure of the energy budget developed here. Figure 7.1 summarises how the next step of this process can be achieved using the advancements presented in this thesis. The search for

biochemical proxies for physiological processes such as growth should be encouraged, and incorporated into this type of modelling approach wherever successful.

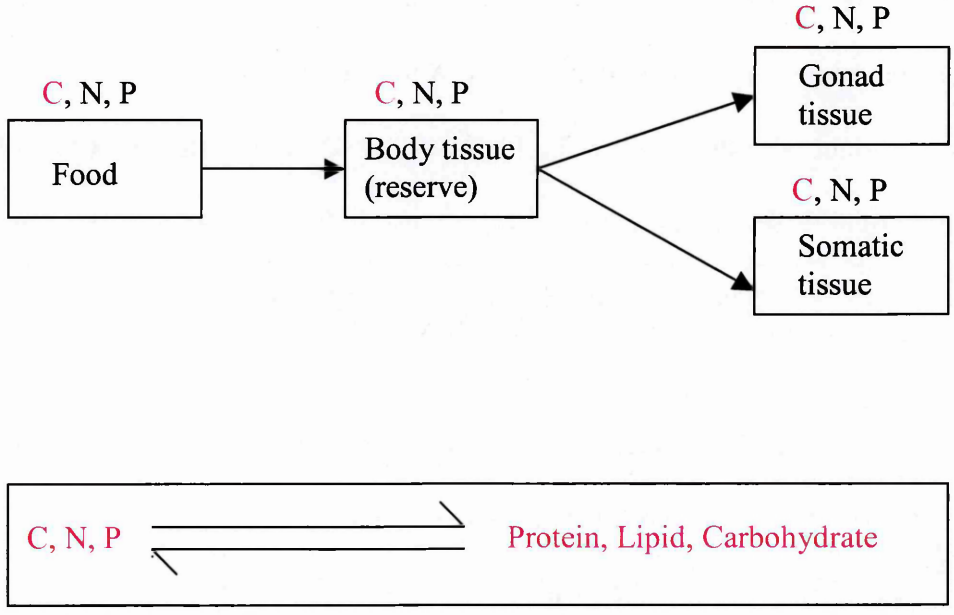

Figure 7.1. The structure for the next step in the development of a predictive energy budget for krill, which uses the developments already achieved by this study (shown in red). Elemental composition of various life history stages of krill is known (Chapter 3) and the model structure exists (Chapter 6) with which carbon is already tracked. This can be extended to track nitrogen and phosphorus similarly and using the stoichiometric conversion (Chapter 5), allow the tracking of the proximate composition of krill at different stages of the model and result in a better understand of the biochemical responses of krill to environmental variability.

Perhaps the most practical, and indeed crucial direction for further work, is the incorporation of the predictive energy budget model for krill growth and reproductive success into large scale ecosystem scale models. The dynamic structure of this model allows for it to be readily embedded into existing ocean transport models, and the outputs in terms of krill biomass and production can feed into models of predator behaviour and response. Ecosystem models are becoming increasingly important to allow us to both to understand better the functioning of the marine ecosystem, and to provide indications of how the ecosystem is likely to change in response to changes in human activities, or climate fluctuation. These models allow scientists to better visualise how species are influenced by each other and by physical conditions.

DeYoung et al. (2004) recognise that difficulties arise in the development of large scale ecosystem models because organisms at higher trophic levels are longer lived and display important variability in abundance and distribution at large spatial and temporal scales. This requires different approaches to that used for modelling phytoplankton and microbes in an ecosystem; individual variability and life-history details become increasingly important with distance up the food web, necessitating structured population and even individual based models to produce meaningful simulations of population dynamics (deYoung et al., 2004). This is where the energetics model of krill developed here becomes important as it allows detailed modelling of individually based life-history strategies at the next level down from top predators. One such ecosystem model, focused on biogeochemistry, is the Dynamic Green Ocean model. The Dynamic Green Ocean Project brings together physical, chemical, biological and paleo-oceanographers with a common interest in modelling and its applications to Earth system problems, to develop a new, more comprehensive model of the oceanic compartment of the Earth system; with a view to improving our understanding of the functioning of the global ocean in the past, present and future. Plans for the development of this model are summarized in Le Quéré et al. (2005). However, as yet this model does not include lifecycle models, such as those proposed by deYoung and co-workers. The inclusion of lifecycle models, such as the one described here for krill, will be the fundamental next step in the development of these models in the future.

The model developed here for krill can obviously be improved with further advances in our knowledge and understanding of krill energetics. Much focused work is still needed in certain aspects of krill energetics, such as feeding strategies in winter, metabolic costs of feeding, Q_{10} responses, and in particular, the cost of swimming. Ensuring that the model is kept up to date with these advances in knowledge, especially if it is being used in a predictive capacity, is essential. The structure of the model allows for

added complexity and detail to easily be incorporated with in the existing structure; for example diurnal migration could be included, as well as mortality.

One particular recommendation of all future work, both chemical and physiological, is that the influence of sexual maturity stage should be specifically included. I would suggest that in future physiological studies, especially those on metabolic rate, that sex and maturity stage be ascribed to each krill, as well as the usual measures such as length and mass, in order to gain a better understanding of the physiological changes that occur with maturity. The results from the chemical composition studies suggest that there is likely to be significant differences in physiological rate processes with changing maturity stage.

The development of the energetics concept presented here is broadly applicable to all organisms, with relatively little adjustment to the structure. I would suggest that this model could, with relatively little work, be developed into a general model for all zooplankton, which would aid its incorporation into ecosystem models, where, at lower levels of complexity, it could be used to represent the entire zooplankton component. Perhaps the first test of the suitability of the model for use on other species would be to use copepods, who as crustacean marine zooplankton like krill, have a similar life-history strategy, which includes moulting, and have been well studied.

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Appendices

APPENDIX A. CHEMICAL AND ELEMENTAL COMPOSITION OF A 'STANDARD' 45MM KRILL.

Table 1. Chemical and elemental composition of a standard krill of 45mm (wet mass 669.99mg, dry mass 172.71mg JR70 and wet mass 689.89mg, dry mass 157.25mg JR82).

Chemical component	Mass per krill (mg)	
	JR70	JR82
Wet mass		
Water	498.47	529.84
Mineral ash	19.36	
Protein	80.40	
Carbohydrate	2.61	
Lipid	43.01	
RNA	1.94	
DNA	0.67	
Chitin	12.46	
Dry mass		
Carbon	83.59	69.51
Hydrogen	12.85	10.63
Nitrogen	16.36	15.85
Phosphorus	2.23	
Mineral ash	19.81	22.44

APPENDIX B. SUMMARY TABLE OF PUBLISHED LENGTH-MASS RELATIONSHIPS FOR KRILL.

Table 1. Length-wet mass relationships. Length given in mm and mass in mg unless otherwise stated in the equation. WM = wet mass, L = length, * = outside predictive range of equation.

Study	Antarctic location	Sex	Equation	Type	Length range	*n*	R^2	Mass (mg) of 45mm krill
Mayzaud et al. (1998b)	Indian sector	All stages	Log_{10} WM (g) = -0.08 + 3.12 log_{10} L	S1	23–44	121	0.967	705.70*
Ju & Harvey (2004)	Marguerite Bay, winter		WM (g) = $0.1x10^{-6}$ $L^{3.478}$	AT	30–62	87	0.991	562.18
Färber-Lorda (1991)	SW Indian Ocean	Subadults	WM = 0.00716 $L^{3.183}$		23–35	161		1309.49*
		MA1	WM = 0.00232 $L^{3.490}$		33–41	45		1365.21*
		MA2	WM = 0.00272 $L^{3.463}$		36–43	24		1444.25*
		Mature females	WM = 0.00587 $L^{3.247}$		33–49	62		1369.68
		Spent females	WM = 0.00170 $L^{3.562}$		34–44	53		1315.81*
		All stages	WM = 0.00503 $L^{3.283}$		23–49	343	0.946	1346.06
Lockyer (1973)			Log_{10} WM = 3.3874 log_{10} L-2.7521	ST1		517		704.68
Kils (1981)		All stages	WM (g) = $1.58x10^{-6}$ $L^{3.40}$	AT		116	0.976	660.05
Sahrhage (1978)			WM (g) = $1.80x10^{-6}$ $L^{3.34}$					598.42
Jadzewski et al. (1978)		Male	WM = 0.0069 $L^{3.0312}$					708.06
		Female	WM = 0.0011 $L^{3.5196}$					724.50
Daly (1990)	Weddell & Scotia Seas, winter	All including furcillia	WM = $1.02x10^{-3}$ $L^{3.49}$		9–50	98	0.99	600.22
Hofmann & Lascara (2000)	Antarctic Peninsula, all year	All stages	WM = 0.0072 $L^{3.021}$		10–40			710.70*
			WM = 0.0016 $L^{3.423}$		40–60			729.57
This study (Equation 7.4)	Scotia Sea, summer	All stages	Log_{10} WM = 3.4403 log_{10} L-2.86494	AT	20–62	615	0.966	664.67

Study	Antarctic location	Sex	Equation	Type	Range	n	R	45mm KRM
This study JR70	South Georgia, summer	All stages	Log_{10} WM = 3.48327 log_{10} L-2.9325	AT	32–62	487	0.959	669.99
This study JR82	Scotia Sea, summer	All stages	Log_{10} WM = 3.43841 log_{10} L-2.8456	AT	20–60	127	0.972	689.89
Siegel (1982a)		All stages	WM (g) = 1.28×10^{-6} $L^{3.45}$	AT	13–60	540		646.83
Siegel (1982b)		All stages	WM (g) = 1.93×10^{-6} $L^{3.33}$	AT	30–62			617.67
Morris et al. (1988)		All stages	WM (g) = 3.85×10^{-6} $L^{3.20}$	AT	34–52	3299	0.81	751.17
		MS1	WM (g) = 10.00×10^{-6} $L^{2.93}$	AT	37–54	106	0.91	698.09
		MS2	WM (g) = 8.13×10^{-6} $L^{2.98}$	AT	43–57	156	0.85	686.54
		MS3	WM (g) = 14.45×10^{-6} $L^{2.84}$	AT	34–57	109	0.80	716.13
		MS	WM (g) = 7.38×10^{-6} $L^{3.01}$	AT	39–57	371	0.90	698.60
		MA1	WM (g) = 12.30×10^{-6} $L^{2.90}$	AT	40–56	387	0.76	765.99
		MA2	WM (g) = 21.38×10^{-6} $L^{2.76}$	AT	34–57	1124	0.73	781.40
		MA	WM (g) = 6.13×10^{-6} $L^{3.08}$	AT	31–48	1882	0.75	757.45
		FS	WM (g) = 29.51×10^{-6} $L^{2.63}$	AT	37–52	77	0.80	657.53
		FA1	WM (g) = 30.20×10^{-6} $L^{2.62}$	AT	37–54	109	0.78	647.77
		FA2	WM (g) = 20.42×10^{-6} $L^{2.73}$	AT	40–56	291	0.77	665.77
		FA3	WM (g) = 29.51×10^{-6} $L^{2.66}$	AT	41–58	398	0.79	737.08
		FA4	WM (g) = 9.77×10^{-6} $L^{2.98}$	AT	39–56	477	0.82	825.03
		FA5	WM (g) = 35.48×10^{-6} $L^{2.59}$	AT	31–58	65	0.73	678.90
		FA	WM (g) = 2.89×10^{-6} $L^{3.27}$	AT	26–59	1417	0.80	736.04

Table 2. Length-dry mass relationships. Length given in mm and mass in mg unless otherwise stated in the equation. DM = dry mass, L = length, †generally smaller than winter specimens.

Study	Antarctic location	Sex	Equation	Type	Length range	*n*	R^2	Mass (mg) of 45 krill
Kato et al. (1982)	64 50 S 120 00 E		$DM = 1.208\ e^{0.104L}$		19–54			130.19
Kils (1981)		All stages	$DM(g) = 6.90x10^{-8}\ L^{3.76}$	AT		114	0.950	113.48
Atkinson et al. (2002)	SW Lazarev Sea Winter	All krill	$Log_{10}\ DM = 3.25\ log_{10}\ L-3.18$	AT	28–38 and 48–58	31	0.978	155.93
Huntley et al. (1994)	Gerlache Straight west Antarctic Peninsula	winter	$Log_{10}\ DW = -3.250 + 3.270\ log_{10}\ L$	S1	15–47	83	0.979	143.22
		summer	$Log_{10}\ DW = -2.909 + 2.866\ log_{10}\ L$		15–47 †	13	0.924	67.47
Atkinson et al. (2006)	South Georgia	Juvenile	$Log_{10}\ DM = 4.09\ log_{10}\ L-4.51$	AT		187	0.81	178.50
		MS1–MS3	$Log_{10}\ DM = 3.67\ log_{10}\ L-3.83$	AT		254	0.82	172.70
		FS	$Log_{10}\ DM = 3.41\ log_{10}\ L-3.38$	AT		32	0.72	180.91
		FA3–FA5	$Log_{10}\ DM = 3.74\ log_{10}\ L-3.90$	AT		38	0.62	191.87
		MA1–MA2	$Log_{10}\ DM = 3.60\ log_{10}\ L-3.76$	AT		19	0.76	155.44
		All stages	$Log_{10}\ DM = 3.89\ log_{10}\ L-4.19$	AT		530	0.89	174.18
This study, both cruises	Scotia Sea, summer	All stages	$Log_{10}\ DM = 3.44685\ log_{10}\ L-3.48163$	AT	22–60	225		164.72
This study JR70	South Georgia, summer	All stages	$Log_{10}\ DM = 3.48253\ log_{10}\ L-3.52004$	AT	36–57	97		172.71
This study JR82	Scotia Sea, summer	All stages	$Log_{10}\ DM = 3.36947\ log_{10}\ L-3.37385$	AT	22–60	127		157.25
Jadzewski et al. (1978)			$DM\ (g) = 0.1x10^{-6}\ L^{3.80}$	AT		145		191.52
Hirche (1983)			$DM = 1.082\ e^{4.009L}$	AT				139.05

Study	Antarctic location	Sex	Equation	Type	Range	n	R	45 km
Morris et al. (1988)		All stages	$DM\ (g) = 1.06 \times 10^{-6}\ L^{3.15}$	AT	31–58	3265	0.67	170.97
		MS1	$DM\ (g) = 0.76 \times 10^{-6}\ L^{3.23}$	AT	34–52	106	0.82	166.22
		MS2	$DM\ (g) = 0.43 \times 10^{-6}\ L^{3.39}$	AT	37–54	156	0.76	172.93
		MS3	$DM\ (g) = 0.09 \times 10^{-6}\ L^{3.80}$	AT	43–57	108	0.73	172.37
		MS	$DM\ (g) = 0.47 \times 10^{-6}\ L^{3.36}$	AT	34–57	370	0.82	168.61
		MA1	$DM\ (g) = 1.35 \times 10^{-6}\ L^{3.08}$	AT	39–57	381	0.72	166.81
		MA2	$DM\ (g) = 6.46 \times 10^{-6}\ L^{2.67}$	AT	40–56	1110	0.64	167.61
		MA	$DM\ (g) = 2.38 \times 10^{-6}\ L^{2.93}$	AT	34–57	1861	0.73	166.15
		FS	$DM\ (g) = 0.87 \times 10^{-6}\ L^{3.20}$	AT	31–48	77	0.66	169.75
		FA1	$DM\ (g) = 4.90 \times 10^{-6}\ L^{2.71}$	AT	37–52	109	0.66	148.05
		FA2	$DM\ (g) = 2.46 \times 10^{-6}\ L^{2.90}$	AT	37–54	290	0.64	153.20
		FA3	$DM\ (g) = 8.13 \times 10^{-6}\ L^{2.65}$	AT	40–56	393	0.67	195.48
		FA4	$DM\ (g) = 2.00 \times 10^{-6}\ L^{3.04}$	AT	41–58	471	0.75	212.23
		FA5	$DM\ (g) = 20.42 \times 10^{-6}\ L^{2.36}$	AT	39–56	64	0.52	162.79
		FA	$DM\ (g) = 0.24 \times 10^{-6}\ L^{3.55}$	AT	37–58	1404	0.69	177.47

APPENDIX C. SUMMARY OF KRILL SAMPLING LOCATIONS FROM JR70 AND JR82.

Table 1. Summary of krill sampling stations during JR70, where chlorophyll *a* concentrations are taken from 6.5 m CTD casts during the cruise. Shelf is defined as 1000m.

Station	Latitude	Longitude	Chl *a* (mg m^{-3})	On/off shelf
30	-53.7065	-36.781	3.48	on
32	-53.7186	-37.1614	12.25	on
33	-53.7177	-37.1797	12.25	on
89	-53.6362	-38.8365	3.47	off
91	-53.6202	-38.9041	3.47	off
111	-53.9386	-38.458	5.54	on
128	-54.0179	-39.138	3.73	on
129	-54.0384	-39.3341	3.52	on
134	-53.8406	-39.1419	1.61	on
137	-53.5765	-39.204	10.15	off
151	-53.3945	-38.7223	12.32	off
152	-53.677	-38.6141	3.57	off
164	-53.3968	-38.031	12.07	off
165	-53.361	-38.0822	12.07	off
168	-54.3127	-35.724	1.25	on
170	-54.301	-35.7435	1.25	on
171	-54.4521	-35.6272	1.29	on
207	-53.3387	-33.7781	0.46	off
252	-53.7965	-36.051	3.48	on
254	-53.7984	-38.1151	3.35	on
276	-53.7963	-38.5129	4.02	on
291	-53.8246	-37.5707	11.04	on
301	-53.5626	-38.6237	3.32	off
302	-53.6483	-37.636	13.46	on
357	-53.7312	-37.4558	12.25	on

Table 2. Summary of krill sampling stations during JR82, where chlorophyll *a* concentrations are taken from 6.5 m CTD casts during the cruise. Shelf is defined as 1000m.

Station	Latitude	Longitude	Chl *a* (mg m^{-3})	On/off shelf	Sea ice
27	-60.3793	-55.0112	0.071	off	—
43	-62.3666	-54.3761	0.056	on	—
49	-62.4342	-53.3671	0.173	off	Ice edge
67	-60.5897	-52.2398	0.154	off	—
85	-59.5827	-51.6662	0.643	off	—
90	-58.5977	-51.0953	0.571	off	—
121	-57.5792	-49.6601	0.076	off	—
144	-59.5439	-48.9648	0.485	off	—
153	-60.5338	-48.6671	0.485	off	—
186	-61.2512	-45.6679	1.151	on	In sea ice
195	-60.0741	-46.9966	0.573	off	—
205	-59.0802	-46.6566	0.172	off	—
215	-58.1081	-46.2757	0.134	off	—
255	-57.9901	-44.8824	0.488	off	—
277	-60.0096	-43.8943	0.987	off	—
292	-60.9161	-43.5079	0.205	on	—
300	-60.7164	-42.0168	0.132	off	—
318	-59.7401	-41.8087	0.146	off	—
331	-58.7414	-41.6785	0.205	off	—
335	-57.7863	-41.4667	0.162	off	—
350	-56.7511	-41.3995	0.147	off	—
440	-57.2975	-36.6552	0.090	off	—
476	-59.746	-33.606	0.975	off	—
498	-61.3381	-31.2905	0.404	off	—
551	-57.4564	-30.4874	—	off	—
571	-56.1519	-32.9063	0.162	off	—
603	-53.7646	-39.2568	—	off	—
604	-53.7667	-39.2459	—	on	—
649	-53.8127	-37.9388	5.968	on	—
676	-53.9786	-36.3141	—	on	—

APPENDIX D. MINITAB ROUTINE TO APPLY STOICHIOMETRIC EQUATIONS OF GNAIGER & BITTERLICH (1984).

```
Gmacro
Chnprox
# This MINITAB macro calculates proximate composition
# from elemental composition (C,H,N and ash, all as
# a percentage of the dry mass); it then estimates energy
# content both from the proximate composition and from
# carbon content
#
# The algorithm is based on the stoichiometric arguments
# in Gnaiger & Bitterlich (Oecologia, 62: 289-298, 1984)
#
# Written by Andrew Clarke
# Version 2, 20th May 1996
# Edited by Kate Arnold 2005 in Minitab v13
Noecho
Note
Note  Proximate composition and energy content macro
Note  (Andrew Clarke, Version 2, May 1996)
Note
Note  Based on Gnaiger & Bitterlich, 1984
Note  (Oecologia, 62: 289-298)
Note
Note
Note  The macro assumes that the data array is organised
Note  as follows:
Note
Note  c1  Reference number
Note  c2  Dry mass (% wet mass)
Note  c3  Ash content (% dry mass)
Note  c4  Carbon (% dry mass)
Note  c5  Nitrogen (% dry mass)
Note  c6  Hydrogen (% dry mass)
Note  c7  Phosphorus  (% dry mass)
Note  c8  Inorganic Carbon (% mineral ash)
#
# A missing value indicator in c1,c2 or c7 is non-fatal
#
# Note that a missing value indicator in any other column (including
# c8, inorganic carbon) will prevent calculation of data for proximate
# composition and energy content; if no data exist for c8, then store
# a value of 0.01 or 0.001
#
# Reference column (c1) cannot be alphanumeric (for this version of
# Minitab, anyway)
#
name  c1 'Ref' c2 'DM(%WM)' c3 'Ash(%DM)' c4 'C(%dryM)' c5 'N(%dryM)'
name  c6 'H(%dryM)' c7 'P(%dryM)' c8 'C(%ash)'
Note
Note  Naming columns and setting defaults
#
# The first step is to set values for three key variables,
# percentage residual water, percentage of non-protein nitrogen
# and the mass-fraction of N in non-protein nitrogen (NPN).
#
#
Note
Note  Enter value for percentage residual water in dry matter
Note  (In the absence of firm data, use the mean value determined
Note  by Gnaiger & Bitterlich, which is 6)
Note
set c90;
file 'terminal';
nobs 1.
let k1=c90(1)
erase c90
Note
Note  Now enter the percentage of total N that is non-protein nitrogen
```

```
Note  (In the absence of firm data, use a value of 5)
Note
set c90;
file 'terminal';
nobs 1.
let k2=c90(1)
erase c90
Note
Note  Finally, enter the mass fraction of N in the non-protein N
Note  (which must lie in the range 0.001 to 1.0); default is 0.15.
Note  The median value for the usual amino acids is 0.15, with a
Note  range from 0.08 to 0.32; the value for chitin is 0.0636
Note  and for nucleic acids the average value is 0.1383
Note
set c90;
file 'terminal';
nobs 1.
let k3=c90(1)
name  k1 '%resWat' k2 'NPN%TotN' k3 'MFNnpn'
erase c90
#
# Now define stoichiometric parameters for protein
#
Let k4=0.173
Let k5=0.529
Let k6=0.070
name  k4 'MFNprot' k5 'MFCprot' k6 'MFHprot'
Note
Note  Calculating organic carbon from residual water and inorganic
carbon
#
# All calculations will be based on absolute mass of
# each element or component in 100 gm dry matter
#
# Now calculate mass of organic carbon, allowing for both
# residual water and inorganic carbon
#
# First, calculate actual dry organic matter (ODM), which is dry
# mass minus both ash (c3) and residual water (k1), and put
# into c9
# Note both are percentages, which is numerically equivalent
# to g/100g; units of c9 are therefore mass (g/100g dry mass)
#
let c9=100-(c3+k1)
name c9 'Org(g)'
#
# Now calculate mass of carbon in ash, which is mass of ash
# (c3, g/100g) times fraction of carbon in ash (c8/100), and
# put into c10; units of c10 are mass (g/100g dry mass)
#
let c10=(c8*c3)/100
name c10 'Cash(g)'
#
# Now calculate organic carbon, which is total carbon (c4)
# minus carbon in ash (c10) and put into c11
# Note units of c11 are mass (g/100g dry mass)
#
let c11=c4-c10
name c11 'Corg(g)'
Note
Note  Calculating protein
#
# Now calculate absolute amount of protein from nitrogen (c5),
# allowing for non-protein nitrogen (k2), and put into c14
# Note units of c14 are mass (g/100g dry mass)
#
# First calculate absolute mass of protein nitrogen (c12)
# and non-protein nitrogen (c13)
#
# Then calculate mass of protein from mass fraction of nitrogen
# in protein (k4); units are g/100 g ODM which is equivalent
# numerically to % dry mass
```

```
#
let c12=c5*(1-(k2/100))
let c13=c5*(k2/100)
let c14=c12/k4
name c12 'Nprot(g)' c13 'Nnpn(g)' c14 'Ptn(%DM)'
#
# Now calculate carbon (c15) and hydrogen (c16) due to this
# protein, units are mass (g/100g ODM)
#
let c15=c14*k5
let c16=c14*k6
name c15 'Cprot(g)' c16 'Hprot(g)'
Note
Note  Calculating fractions of lipid and carbohydrate
#
# Now calculate organic matter remaining after allowing for
# protein (c17, units g/100g ODM), and estimate mass fraction
# of carbon in this (c18, dimensionless)
#
let c17=c9-c14
let c18=(c11-c15)/c17
name c17 'ODM-Ptn' c18 'MFC(kl)'
#
# Now use the mass fraction of carbon in the non-protein organic
# matter to estimate the proportion of lipid and carbohydrate
#
# The mass fraction of carbon in lipid (k7) and carbohydrate (k8)
# are 0.776 and 0.444 respectively; MFC(kl) (c18) can be used
# to estimate the proportion of lipid (c19, scale 0-1) and non-lipid
# (carbohydrate plus NPN) (c20, scale 0-1) in non-protein organic
# matter by:
#
# c19 = (c18-0.444)/(0.776-0.444), and
# c20 = 1-c19
#
# Note that errors in the estimation of protein can lead to values
# of c19>1 (when MFC(kl)>0.776) and hence to negative values for c20
#
# First define the stoichiometry of lipid and carbohydrate
#
let k7=0.776
let k8=0.444
let k9=0.114
let k10=0.062
name k7 'MFClip' k8 'MFCcarb' k9 'MFHlip' k10 'MFHcarb'
#
# Now calculate proportions of lipid and carbohydrate in non-protein
# organic matter
#
let c19=(c18-k8)/(k7-k8)
let c20=1-c19
name c19 'propLip' c20 'prNonLip'
#
# Now calculate absolute masses (units g/100g ODM) of lipid and
# non-lipid in the non-protein organic matter (c21, c22); again
# these are equivalent numerically to % dry mass
#
let c21=c19*c17
let c22=c20*c17
name c21 'Lip(%DM)' c22 'Non-lip'
#
# Now estimate the carbon (c23, c25) and hydrogen (c24, c26)
# explained by these components
#
let c23=c21*k7
let c24=c21*k9
let c25=c22*k8
let c26=c22*k10
name c23 'Clip(g)' c24 'Hlip(g)' c25 'CNonL(g)' c26 'HNonL(g)'
Note
Note  Calculating non-protein nitrogen component
#
```

```
# Now estimate the mass of the non-protein nitrogenous component (c27)
# from the mass of non-protein nitrogen (c13) and the mass fraction of
# nitrogen in NPN (k3); as before, the units of c27 are g/100 g ODM,
# which is equivalent numerically to % dry mass:
#
let c27=c13/k3
name c27 'NPN(%DM)'
#
# Now estimate the carbohydrate (c28, units g/100g ODM) by subtracting
# the mass of NPN (c27) from that of the non-lipid protein-free ODM
# (c22)
#
let c28=c22-c27
name c28 'Carb(g)'
Note
Note  Calculating unexplained component
#
# Now estimate amount of unexplained organic matter (g/100g ODM)
# and place in c29; as before the units are numerically
# equivalent to % dry mass:
#
# This calculation uses calculated amounts of carbohydrate and
# NPN, including negative values where relevant
# (Error term 2 calculates unexplained material with absolute
# values of lipid and carbohydrate)
#
let c29=c9-(c14+c21+c27+c28)
name c29 'Unexp(%)'
Note
Note  Calculating unexplained hydrogen and residual water
#
# Now estimate amount of unexplained hydrogen (units g/100g ODM)
# and place in c30
#
# Note that c30 excludes the hydrogen due to the residual water
# but includes all stochastic errors
#
let c30=c6-(c16+c24+c26)
name c30 'Hres(g)'
#
# Now calculate estimated residual water (c31), though note that this
# calculation includes all stochastic and analytical errors)
#
# Residual water is calculated simply from the molecular weight of water
# (18.0158) and the relative weight of the hydrogen component
# (2*1.00794); the units are g/100g ODM (equivalent to % dry mass)
#
# The degree of error can be estimated by subtracting the residual
# water set at the start (k1)
#
let c31=c30*(18.0158/2.0159)
let c32=c31-k1
name c31 'Water(g)' c32 'Err1(g)'
Note
Note  Calculating error terms
#
# A second measure of error may be calculated from the absolute
# levels of lipid and carbohydrate
#
let c34=absolute(c21)
let c35=absolute(c22)
let c33=c9-(c14+c34+c35)
name c33 'Err2(g)'
erase c34:c35
Note
Note  Negative values of lipid occur when carbon explained by protein
Note  has been overestimated (MFCkl>0.776)
Note
Note  Negative values of carbohydrate occur when carbon explained by
Note  protein has been underestimated (MFCkl<0.444)
print c1,c29,c31,c32,c18,c33
Note  Unexplained matter term (% dry mass) is calculated from actual
```

```
Note  values of lipid and carbohydrate, including negative values
Note  where these were found
Note
Note  Error term 1 is estimated residual water minus set residual water;
a
Note  value close to zero means that residual water has been correctly
Note  estimated, although all stochastic errors will accumulate in the
Note  residual hydrogen term used to calculate this error term
Note
Note  Error term 2 is unexplained matter calculated from protein, ash
and
Note  non protein nitrogen, plus absolute values of lipid and
carbohydrate
Note
Note  MFCkl should lie between 0.776 and 0.444
Note
Note  If MFCkl is >0.776, then lipid will be overestimated and
Note  carbohydrate values will be negative; the most likely explanations
Note  for this are that the fraction of non-protein nitrogen has been
Note  overestimated, or that residual water values are too high
Note
Note  If MFCkl is <0.444, then carbohydrate will be overestimated and
Note  lipid values will be negative; the most likely explanation for
this
Note  is a severe underestimation of residual water
Note
Note
Note  Calculating energy content of tissues
#
# Now calculate energy content (enthalpy of combustion) from
# proximate composition, and place in c35
#
# Note that units will be kJ/g (dry mass), once corrected
# for the calculation based on 100 g dry matter
#
# First set enthalpies of combustion for protein, lipid,
# carbohydrate and NPN (assume this to be carbohydrate)
#
let k11=-23.9
let k12=-39.5
let k13=-17.5
name k11 'kJ/gProt' k12 'kJ/gLip' k13 'kJ/gCH2O'
#
# Now calculate energy content from proximate composition
#
let c34=((c14*k11)+(c21*k12)+(c22*k13))/100
name c34 'kJ/gDM-P'
#
# Now calculate energy content from carbon content, which is based on
# an empirical relationship between enthalpy of combustion and the
# mass fraction of carbon in the organic matter (not corrected for
# residual water; the residual water is corrected for in the
# calculation of energy content
#
# First calculate the mass fraction of carbon in organic matter
# and place into c35 (c11 is carbon corrected for carbon in ash)
#
let c35=c11/(100-c2)
name c35 'MFCorg'
#
# Now calculate energy content from Gnaiger & Bitterlich expression
# and place into c36; units are kJ/g
#
let k14=(100-k1)/100
name k14 'MFwater'
let c36=(11.49*k14)-(66.27*c35)
name c36 'kJ/gDM-C'
print c1,c34,c36
Note  kJ/gDM-P is energy content estimated from proximate composition
Note  It uses standard enthalpies of combustion for protein, lipid and
Note  carbohydrate, and assumes that the non-protein nitrogen component
Note  is a carbohydrate
```

```
Note
Note  kJ/gDM-C is the energy content estimated from organic carbon
Note  (Where the estimate of proximate composition is equivocal, then
Note  this is the better estimate of energy content)
Note
Note  Both estimates are expressed as kJ per gram dry mass
Note  (The dry mass figure includes the residual water)
Note
Note  Energy contents are expressed in negative terms as this is the
Note  thermodynamic convention for an exothermic reaction
Note
Endmacro
```

APPENDIX E. MATHEMATICAL CODE FOR THE ENERGETICS MODEL.

```
%Model structure for krill growth. Initially constructed with parameters
%for krill at South Georgia.

%Parameters

Q10 = 2.62;
assimilation_efficiency = 0.8;
start_repro = 29.2295; %carbon equivalent to a length of 33mm
allocation_to_reproduction = 0.7;
cost_of_growth = 0.3;
repro_decay = 0.02;
prev_IMP = 0;

%Initial values:

t = 1;

krill_carbon(1) = 7.6747; %for juvenille krill, inital length of 22mm

somatic_store = krill_carbon;
oc1_oc2(1) = 0;
oc3_oc4(1) = 0;
z(1) = 1; %index for moulting period
y(1) = 1; %index for spawning period
spawn_count=0;
spawn_total=0;
moult_total=0;

%Load functions already saved
load time_temp
load SG_temp
load time_food
load SG_food
load pp_drymass_filt

%adjustment for day model run starts (Julian day number)
t_start = 1; %J day number for t=1
t_end = t_start+364;
time = 1:1:365;
new_time = t_start:1:t_end;

pp_time_temp = spline(time_temp,SG_temp);
new_temp = ppval(pp_time_temp,new_time);
pp_time_temperature = spline(time,new_temp);

pp_time_fd = spline(time_food,SG_food);
new_food = ppval(pp_time_fd,new_time);
pp_time_food = spline(time,new_food);

%Model

k = 1; %index for output vectors
for yr = 1:2
    for t = 1:365

        %if t>120&t<=280
            %winter_food(k) = 50;
```

```
        %else
            %winter_food(k) = 0;
        %end;

        temperature(k) = ppval(pp_time_temperature,t);
        %temperature(k) = 1;
        food(k) = ppval(pp_time_food,t);%+winter_food(k);
        %food(k) = 75;

        dry_mass(k) = (krill_carbon(k)+2.24989)/0.502455;
        wet_mass(k) = (dry_mass(k)-5.61271)/0.249439;
        spawn(k) = (krill_carbon(k)*0.5)/2;
        length(k) = (wet_mass(k)/0.001365).^(1/3.4403);


        %metabolism
        maintenance_cost(k) = (10^(0.0285*length(k)-
2.7873)*Q10^((temperature(k)--1.5)/10))*0.5357*24;
        swimming_cost(k) = (krill_carbon(k)/100)*0.5;
        metabolic_cost(k) = maintenance_cost(k)+swimming_cost(k);

        %feeding
        filtration_rate(k) = ppval(pp_drymass_filt,dry_mass(k));

        if krill_carbon(k)<1.077 %t>120&t<=280
            time_feeding(k) = 0.9;
        else
            time_feeding(k) = 0.75;
        end;

        ingestion(k) = filtration_rate(k)*food(k)*time_feeding(k);
        assimilation(k) = ingestion(k)*assimilation_efficiency;
        feeding_cost(k) = maintenance_cost(k)*0.01*time_feeding(k);
        carbon_post_feed(k) = (assimilation(k)-metabolic_cost(k)-
feeding_cost(k));

        %partition of energy into growth or reproduction
        if krill_carbon(k)>=start_repro&carbon_post_feed(k)>0
            somatic_growth(k) = carbon_post_feed(k)*(1-
allocation_to_reproduction);
        else
            somatic_growth(k) = carbon_post_feed(k);
        end;

        somatic_cost(k) = somatic_growth(k)*cost_of_growth;
        somatic_carbon(k) = somatic_growth(k)-somatic_cost(k);

        if krill_carbon(k)>=start_repro&carbon_post_feed(k)>0
            repro_growth(k) =
carbon_post_feed(k)*allocation_to_reproduction;
        else
            repro_growth(k) = 0;
        end;

        repro_cost(k) = repro_growth(k)*cost_of_growth;
        repro_carbon(k) = repro_growth(k)-repro_cost(k);

        %moulting
        IMP(k) = (-3.04*temperature(k))+23.21; %Buccholtz
        %IMP(k) = exp(3.5371-(0.5358*log(temperature(k)+2))); %Kawaguchi
```

```
        if z(k)/IMP(k)>=1
            moult_loss(k) = (krill_carbon(k)/100)*2.05;
            z(k)=0;
            prev_IMP=IMP(k);
            moult_total(k+1)=moult_total(k)+moult_loss(k);
        else
            moult_loss(k) = 0;
            moult_total(k+1)=moult_total(k)+0;
        end;

        %development of the ovaries
        if repro_carbon(k)>0 & oc1_oc2(k)<spawn(k)
            oc1_oc2(k) = oc1_oc2(k)+repro_carbon(k);
            oc3_oc4(k) = oc3_oc4(k) + 0;
        elseif repro_carbon(k)>0 & oc1_oc2(k)>=spawn(k)
            oc1_oc2(k) = oc1_oc2(k)+0;
            oc3_oc4(k) = oc3_oc4(k)+repro_carbon(k);
        end;

        %spawning
        spawn_IMP(k) = (IMP(k)+prev_IMP)-5;

        if y(k)/spawn_IMP(k)>=1 & oc3_oc4(k)>0
            spawn_count(k+1) = spawn_count(k)+1;
        else spawn_count(k+1) = spawn_count(k)+0;
        end;

        if  y(k)/spawn_IMP(k)>=1
            y(k)=0;
            spawned_carbon(k)= oc3_oc4(k);
            oc3_oc4(k) = 0;
            spawning(k) = k;
            spawn_total(k+1)=spawn_total(k)+spawned_carbon(k);
        else spawned_carbon(k)=0;
            spawn_total(k+1)=spawn_total(k)+0;
        end;



        %winter re-absorbtion of remaining ovarian tissue
        if t>120&t<=220 & oc3_oc4(k)>0 %reabsorb ovarian tissue in winter
            somatic_total(k) = (somatic_carbon(k)-
moult_loss(k))+(repro_decay*oc3_oc4(k));
            decay_oc3_oc4(k) = repro_decay*oc3_oc4(k);
            decay_oc1_oc2(k)=0;
            repro_carbon(k)= repro_carbon(k)-decay_oc3_oc4(k);
        elseif t>120&t<=220 & oc3_oc4(k)<=0
            somatic_total(k) = (somatic_carbon(k)-
moult_loss(k))+(repro_decay*oc1_oc2(k));
            decay_oc1_oc2(k) = repro_decay*oc1_oc2(k);
            decay_oc3_oc4(k)=0;
            repro_carbon(k)= repro_carbon(k)-decay_oc1_oc2(k);
        else
            somatic_total(k) = somatic_carbon(k)-moult_loss(k);
            decay_oc1_oc2(k) = 0;
            decay_oc3_oc4(k)=0;
        end;

        oc1_oc2(k)=oc1_oc2(k)-decay_oc1_oc2(k);
        oc3_oc4(k)=oc3_oc4(k)-decay_oc3_oc4(k);
        ovary(k+1) = oc1_oc2(k)+oc3_oc4(k);
        oc1_oc2(k+1) = oc1_oc2(k);
```

```
        oc3_oc4(k+1) = oc3_oc4(k);

        somatic_intermediate(k) = somatic_store(k)+somatic_total(k);
        dry_mass_store(k) = (somatic_intermediate(k)+2.24989)/0.502455;
        wet_mass_store(k) = (dry_mass_store(k)-5.61271)/0.249439;
        krill_somatic_length(k) =
((wet_mass_store(k)/1000)/0.00000613).^(1/3.08);

        net_carbon(k) = somatic_total(k)+repro_carbon(k)-
spawned_carbon(k);
        percentage_carbon(k) = net_carbon(k)/krill_carbon(k)*100;
        metabolism(k) =
metabolic_cost(k)+feeding_cost(k)+somatic_cost(k)+repro_cost(k);

        if k>1
            growth_rate(k) = length(k) - length(k-1);
            somatic_growth_rate(k) = krill_somatic_length(k) -
krill_somatic_length(k-1);
            carbon_growth_rate(k)=krill_carbon(k) - krill_carbon(k-1);
        end;

        z(k+1) = z(k)+1;
        y(k+1) = y(k)+1;
        somatic_store(k+1)=somatic_intermediate(k);
        krill_carbon(k+1) = krill_carbon(k)+net_carbon(k);
        test_carbon(k)= somatic_store(k)+ovary(k)-krill_carbon(k);
        spawn_percentage(k)=spawned_carbon(k)/krill_carbon(k)*100;
        k = k+1;

        end;

end;
```

APPENDIX F. LENGTH RANGES OF KRILL CAUGHT DURING JR70 AND JR82.

Table 1. Range of lengths of krill from varying sexual maturity stages collected from three different studies.

Sexual maturity	JR70			JR82			Morris et al. (1988)	
stage	Range	Mean	*n*	Range	Mean	*n*	Range	*n*
J	32–47	38.5	68	20–44	31.8	58	—	—
FS	33–47	40.9	100	42	42.0	1	31–48	77
FA1	46–51	48.5	2	33–54	43.7	33	37–52	109
FA2	48–54	51.3	4	47–52	49.3	3	37–54	289
FA3	50–56	52.8	15	49–56	53.3	3	40–56	398
FA4	51–62	57.0	50	49–58	55.1	13	41–59	477
FA5	—	—	—	49–60	55.0	10	39–56	65
MS1	34–47	41.3	89	33	33.0	1	34–52	105
MS2	40–53	45.1	75	48	48	1	37–54	155
MS3	47–54	50.1	14	—	—	—	43–57	108
MA1	36–56	52.3	24	45–47	46.0	2	39–57	383
MA2	50–61	54.4	46	48–56	52.7	3	40–56	1113

APPENDIX G. SENSITIVITY ANALYSIS.

Table 1. Sensitivity analysis run varying one parameter or variable in isolation (others as described in Table 6.4) by ± 10% (and maximum and minimum values where appropriate) for a two year model run. Data shown is percentage difference from original total krill carbon value from original model run.

	Time (days)															
	25	50	100	150	200	250	300	350	400	450	500	550	600	650	700	730
IMP																
Kawaguchi et al. (2006)	0.00	-0.03	2.48	1.45	-0.14	-0.14	1.46	-3.41	-2.92	-0.41	3.21	1.54	1.56	1.62	18.82	23.76
Quetin et al. (1994)	0.00	-0.03	1.35	-0.26	-0.27	-0.29	-0.30	3.38	4.24	-0.07	1.89	0.26	0.26	0.27	6.94	8.58
Tarling et al (2006) J	-0.02	1.35	6.95	4.02	2.59	-0.53	-2.12	-7.75	16.59	1.16	4.58	1.25	-0.36	-3.54	-11.50	-6.09
Tarling et al (2006) FS	-0.01	1.38	3.99	2.20	2.37	2.59	2.71	9.30	10.65	0.74	2.61	0.97	-0.63	-0.61	16.93	20.57
Tarling et al (2006) FA	-1.42	-1.20	0.71	-1.21	-7.68	-12.93	-18.85	-12.04	3.07	8.35	2.24	-1.01	-5.70	-10.36	-3.92	-14.55
+ 10%	-0.01	-0.05	2.01	1.22	1.30	1.46	3.18	-7.75	-9.05	-0.61	3.33	3.29	3.32	3.42	20.13	22.12
- 10%	0.02	-1.43	-1.21	-1.88	-3.62	-3.92	-2.57	6.00	-4.23	-6.32	-2.12	-3.64	-3.70	-5.34	8.71	9.44
Q_{10}																
1.1	6.99	11.84	12.59	19.95	28.12	36.73	44.85	18.51	14.75	18.06	23.80	29.89	34.71	38.95	30.96	28.40
8.5	-14.03	-24.51	-25.14	-38.86	-49.57	-59.13	-65.55	-54.39	-28.09	-33.34	-41.52	-48.52	-55.18	-61.42	-49.87	-33.70
+ 10%	-0.92	-1.59	-0.10	-1.19	-2.02	-2.81	-3.51	-1.27	0.22	-0.89	-1.60	-2.25	-2.77	-3.29	-4.54	-2.62
- 10%	0.98	1.69	2.25	3.32	4.39	5.49	6.44	3.12	3.02	3.19	3.89	4.60	5.19	5.82	4.46	3.87
Assimilation																
0.704	-11.26	-20.71	-13.69	-16.94	-19.90	-23.91	-29.47	-29.22	-20.25	-21.01	-21.91	-23.26	-25.56	-29.32	-24.12	-21.97
0.946	19.20	35.74	23.13	27.57	32.66	40.88	55.72	35.55	34.48	33.82	35.36	37.69	40.80	46.51	46.16	47.26
+ 10%	10.19	19.09	13.20	15.68	18.50	22.83	30.12	18.51	18.49	17.45	18.30	19.55	21.24	24.63	24.19	25.44
- 10%	-9.45	-17.47	-11.02	-13.73	-16.21	-19.60	-24.45	-22.84	-17.50	-18.11	-18.85	-19.97	-21.90	-25.12	-22.06	-19.49
Allocation to repro																
0.5	0.00	0.00	14.18	13.30	14.22	15.64	16.50	16.13	32.47	43.27	41.32	40.49	40.24	39.86	40.09	48.60
+ 10%	0.00	0.00	-5.10	-4.92	-5.30	-5.83	-6.11	-5.16	-9.17	-13.24	-13.14	-13.01	-13.30	-13.84	-13.70	-15.68
- 10%	0.00	0.00	5.26	4.98	5.34	5.88	6.18	5.07	12.02	15.13	14.74	14.52	14.55	14.81	15.48	18.28
Kg																
+ 10%	-2.91	-5.70	-1.07	-0.37	1.01	2.40	2.64	-2.27	-2.42	-2.67	-1.83	-0.62	0.53	1.27	-2.03	-1.62
- 10%	2.97	5.87	2.66	1.85	0.57	-0.65	-0.81	3.53	4.63	3.98	3.04	1.77	0.62	-0.07	1.22	2.27

	Time (days)															
	25	50	100	150	200	250	300	350	400	450	500	550	600	650	700	730
Reproductive decay																
0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.50	3.55	2.81	2.71	2.68	2.71	2.78	7.62	7.50
+ 10%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.04	1.02	0.69	0.67	0.66	0.67	0.69	1.89	1.54
- 10%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.29	0.04	0.04	0.04	0.04	0.04	-1.14	-0.94
Start reproduction																
+ 10%	0.00	0.00	5.44	5.15	5.52	6.07	6.39	6.08	5.29	4.53	4.37	4.31	4.35	4.47	3.24	2.62
- 10%	0.00	0.00	-3.80	-3.66	-3.94	-4.33	-4.54	-4.83	-3.20	-3.09	-2.99	-2.95	-2.99	-3.09	-2.02	-1.64
38 mm	0.00	0.00	19.20	17.95	19.15	21.05	22.24	23.85	35.88	32.34	30.84	30.28	30.16	30.20	39.43	35.80
33mm	0.00	0.00	10.75	10.12	10.84	11.92	12.56	13.85	9.86	8.61	8.29	8.18	8.22	8.42	5.46	5.70
Temperature																
-1.5	9.23	15.78	26.60	32.22	42.12	55.56	68.44	36.43	41.62	39.10	52.43	59.73	65.47	68.95	70.36	99.56
0	5.25	10.51	27.15	20.84	25.54	29.53	34.41	34.08	9.89	20.42	31.15	34.39	35.84	34.19	32.09	66.34
2	1.67	5.14	13.03	10.00	5.97	1.05	-5.15	-8.52	17.49	5.56	9.05	5.61	0.23	-6.14	11.30	17.67
5	-6.40	-9.11	-11.08	-21.38	-39.01	-57.68	-72.56	-63.67	-34.35	-33.31	-36.28	-47.33	-60.15	-73.31	-66.81	-47.09
+ 10%	-0.60	-2.55	-1.56	-2.43	-2.91	-3.32	-2.03	-11.32	-5.20	-6.22	-2.59	-4.41	-4.60	-4.81	13.41	11.86
- 10%	0.58	1.01	3.88	3.35	2.22	2.67	4.60	12.53	10.37	-0.60	4.15	2.79	2.96	3.11	-1.09	1.27
Food																
150	72.52	88.53	170.38	522.25	1089.56	2177.08	2813.51	780.62	493.57	531.88	752.25	1178.41	1913.78	2606.51	1284.61	948.99
100	5.99	-9.56	56.56	221.15	493.25	1019.87	1326.41	330.29	192.18	212.29	322.94	531.50	887.88	1210.17	567.59	405.26
50	-38.92	-67.89	-54.75	-13.24	91.33	266.40	359.98	39.21	-8.35	-5.46	24.15	80.48	175.78	262.92	85.27	40.04
20	-58.56	-85.56	-91.48	-93.31	-93.31	-93.00	-93.25	-98.04	-99.04	-99.20	-98.90	-98.20	-97.12	-96.05	-97.93	-98.57
+ 10%	10.19	19.09	13.20	15.68	18.50	22.83	30.12	18.51	18.49	17.45	18.30	19.55	21.24	24.63	24.19	25.44
- 10%	-9.45	-17.47	-11.02	-13.73	-16.21	-19.60	-24.45	-22.84	-17.50	-18.11	-18.85	-19.97	-21.90	-25.12	-22.06	-19.49
Spawn																
+ 10%	0.00	0.00	3.12	2.96	3.18	3.49	3.68	2.50	4.13	3.45	3.34	3.29	3.32	3.42	4.04	5.98
- 10%	0.00	0.00	-2.91	-2.80	-3.02	-3.32	-3.48	-2.39	-2.73	-3.65	-3.53	-3.49	-3.54	-3.66	-4.05	-4.73
Maintenance cost																
+ 10%	-2.18	-3.48	-2.59	-5.37	-8.67	-12.58	-16.31	-7.31	-4.89	-5.82	-7.45	-9.67	-12.19	-15.10	-12.14	-9.29
- 10%	2.21	3.51	4.51	7.28	10.99	15.60	20.27	7.33	5.10	5.81	7.61	10.12	12.83	15.95	12.80	12.44

		Time (days)															
		25	50	100	150	200	250	300	350	400	450	500	550	600	650	700	730
RQ																	
	0.72	6.24	9.91	10.42	17.98	28.78	42.55	57.24	22.00	14.73	16.94	22.40	30.23	38.64	47.34	38.21	34.42
	0.97	0.66	1.05	1.42	2.24	3.33	4.67	5.99	2.87	2.66	2.46	2.96	3.68	4.48	5.42	4.20	4.88
Swimming cost																	
	+ 10%	-0.76	-1.39	-0.06	-1.79	-3.71	-5.84	-7.77	-3.28	-2.04	-2.85	-4.01	-5.64	-7.39	-9.31	-9.79	-7.12
	- 10%	0.76	1.41	2.10	3.79	5.99	8.57	10.98	4.87	4.51	4.57	5.76	7.56	9.52	11.69	9.46	9.46
Filtration rate																	
	+ 10%	10.19	19.09	13.20	15.68	18.50	22.83	30.12	18.51	18.49	17.45	18.30	19.55	21.24	24.63	24.19	25.44
	- 10%	-9.45	-17.47	-11.02	-13.73	-16.21	-19.60	-24.45	-22.84	-17.50	-18.11	-18.85	-19.97	-21.90	-25.12	-22.06	-19.49
Time feeding																	
	+ 10%	10.17	19.06	13.17	15.62	18.41	22.71	29.96	18.40	18.40	17.36	18.20	19.43	21.10	24.46	24.07	25.34
	- 10%	-9.44	-17.44	-10.98	-13.68	-16.12	-19.49	-24.33	-22.72	-17.41	-18.02	-18.75	-19.86	-21.77	-24.97	-21.95	-19.39
Feeding cost																	
	+ 10%	-0.02	-0.03	-0.03	-0.05	-0.08	-0.12	-0.15	-0.12	-0.09	-0.09	-0.10	-0.12	-0.14	-0.16	-0.13	-0.11
	- 10%	0.01	0.03	0.03	0.06	0.08	0.11	0.15	0.12	0.09	0.09	0.10	0.12	0.14	0.16	0.13	0.11
Moult loss																	
	+ 10%	-0.15	-0.40	1.02	0.49	0.19	-0.14	-0.63	-0.65	-0.76	-1.42	-1.99	-2.28	-2.63	-3.03	-4.35	-2.50
	- 10%	0.15	0.40	1.03	1.47	1.92	2.46	3.09	0.57	1.26	1.48	2.06	2.37	2.72	3.14	2.51	3.60

Table 2. Sensitivity results for varying the model parameters and variables, whilst temperature and food are held constant at 1 °C 75 mg carbon m^{-3}.

Parameter	Growth rate at day 2 (mg carbon day^{-1})			Growth rate at day 100 (mg carbon day^{-1})			Number of spawns			Total carbon spawned (mg carbon)			Length at day 100 (mm)		
	20	40	60	20	40	60	20	40	60	20	40	60	20	40	60
Q_{10}															
1	0.186	0.844	0.956	0.821	1.004	0.794	0	2	0	0	26.81	0	39.15	49.69	64.94
1.5	0.180	0.822	0.875	0.791	0.968	0.709	0	2	0	0	16.48	0	38.70	50.55	64.41
2	0.176	0.805	0.813	0.768	0.934	0.647	0	1	0	0	15.36	0	38.35	50.37	64.01
2.5	0.172	0.792	0.761	0.750	0.906	0.599	0	1	0	0	14.97	0	38.05	50.17	63.68
3	0.169	0.780	0.716	0.734	0.883	0.558	0	1	0	0	14.65	0	37.80	49.99	63.40
3.5	0.166	0.769	0.677	0.720	0.863	0.524	0	1	0	0	13.75	0	37.57	49.91	63.16
4	0.163	0.760	0.642	0.708	0.845	0.494	0	1	0	0	13.51	0	37.36	49.77	62.94
4.5	0.161	0.751	0.610	0.698	0.829	0.467	0	1	0	0	13.28	0	37.17	49.64	62.74
5	0.159	0.743	0.580	0.688	0.814	0.443	0	1	0	0	12.51	0	36.99	49.59	62.56
Time feeding															
0.5	0.084	0.374	-0.082	0.200	0.358	-0.003	0	1	0	0	1.769	0	28.64	45.36	58.58
0.55	0.102	0.457	0.085	0.288	0.453	0.105	0	1	0	0	1.937	0	30.52	46.66	59.62
0.6	0.119	0.540	0.251	0.397	0.557	0.219	0	1	0	0	4.722	0	32.44	47.57	60.64
0.65	0.136	0.623	0.417	0.520	0.667	0.337	0	1	0	0	7.933	0	34.36	48.44	61.64
0.7	0.154	0.706	0.583	0.639	0.781	0.460	0	1	0	0	11.39	0	36.23	49.28	62.63
0.75	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
0.8	0.189	0.871	0.916	0.860	1.025	0.721	0	2	0	0	18.88	0	39.65	50.91	64.57
0.85	0.206	0.954	1.082	0.980	1.155	0.858	0	2	0	0	22.61	0	41.22	51.74	65.52
0.9	0.223	1.037	1.249	1.108	1.290	1.000	0	2	0	0	26.75	0	42.73	52.52	66.45
0.95	0.241	1.120	1.415	1.23	1.430	1.146	1	2	0	1.472	30.25	0	43.89	53.38	67.37
1	0.258	1.203	1.581	1.36	1.575	1.297	1	2	0	3.995	35.10	0	44.85	54.10	68.29
Assimilation efficiency															
0.704	0.140	0.639	0.450	0.545	0.689	0.360	0	2	0	0	14.45	0	34.73	47.82	61.84
0.754	0.156	0.717	0.606	0.653	0.797	0.477	0	1	0	0	11.61	0	36.48	49.42	62.77
0.804	0.172	0.795	0.762	0.754	0.910	0.598	0	1	0	0	15.03	0	38.12	50.20	63.68
0.854	0.189	0.873	0.919	0.861	1.027	0.723	0	2	0	0	18.91	0	39.67	50.93	64.59
0.904	0.205	0.950	1.075	0.974	1.149	0.852	0	2	0	0	22.50	0	41.15	51.69	65.48
0.954	0.221	1.028	1.231	1.094	1.275	0.985	0	2	0	0	26.47	0	42.57	52.42	66.36
Slope of metabolism															
0.0250	0.182	0.860	1.120	0.833	1.064	1.022	0	2	0	0	28.86	0	39.11	49.94	66.14
0.0268	0.177	0.827	0.957	0.791	0.997	0.813	0	2	0	0	16.81	0	38.59	50.70	65.00

Parameter	Growth rate at day 2 (mg carbon day^{-1})			Growth rate at day 100 (mg carbon day^{-1})			Number of spawns			Total carbon spawned (mg carbon)			Length at day 100 (mm)		
	20	40	60	20	40	60	20	40	60	20	40	60	20	40	60
0.0285	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
0.0303	0.165	0.743	0.486	0.697	0.793	0.361	0	1	0	0	12.30	0	37.31	49.49	61.95
0.0320	0.159	0.690	0.150	0.646	0.677	0.149	0	1	0	0	9.801	0	36.55	48.71	60.02
Intercept of metabolism															
2.500	0.106	0.545	-0.156	0.393	0.507	-0.008	0	1	0	0	8.276	0	31.95	46.67	58.32
2.644	0.144	0.687	0.371	0.617	0.716	0.288	0	1	0	0	10.15	0	35.67	48.90	61.30
2.787	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
2.931	0.191	0.861	1.022	0.846	1.050	0.867	0	2	0	0	18.34	0	39.51	51.05	65.37
3.075	0.205	0.914	1.217	0.924	1.167	1.109	0	2	0	0	21.55	0	40.54	51.66	66.69
Cost of growth															
0	0.244	1.126	1.071	1.187	1.286	0.721	1	2	0	10.23	41.12	0	42.67	51.41	65.01
0.1	0.220	1.014	0.964	1.065	1.155	0.684	0	2	0	0	24.02	0	42.50	51.95	64.57
0.2	0.2	0.901	0.857	0.904	1.029	0.640	0	2	0	0	19.19	0	40.34	51.08	64.11
0.3	0.200	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
0.4	0.147	0.676	0.643	0.587	0.770	0.529	0	1	0	0	10.58	0	35.40	49.11	63.07
0.5	0.122	0.563	0.535	0.407	0.638	0.462	0	1	0	0	6.567	0	32.66	48.02	62.49
0.6	0.098	0.451	0.428	0.255	0.505	0.386	0	1	0	0	2.427	0	29.95	46.90	61.87
Reproductive decay															
0	0.171	0.788	0.750	0.746	0.898	0.588	0	2	0	0	22.98	0	37.99	49.11	63.61
0.01	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
0.02	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
0.03	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
0.04	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
0.05	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
Start reproduction															
30.0	0.171	0.788	0.750	0.727	0.898	0.588	1	2	0	3.309	22.98	0	37.13	49.11	63.61
31.2	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
32.3	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
33.3	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
34.3	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
35.2	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
36.0	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
36.8	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61

Parameter	Growth rate at day 2 (mg carbon day^{-1})			Growth rate at day 100 (mg carbon day^{-1})			Number of spawns			Total carbon spawned (mg carbon)			Length at day 100 (mm)		
	20	40	60	20	40	60	20	40	60	20	40	60	20	40	60
38.3	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
38.9	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
39.6	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
40.2	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	13.67	0	37.99	50.27	63.61
Intermoult period															
8	0.171	0.788	0.750	0.724	0.880	0.678	0	6	0	0	31.80	0	36.95	46.45	61.83
13	0.171	0.788	0.750	0.738	0.895	0.624	0	3	0	0	23.47	0	37.60	48.51	62.94
18	0.171	0.788	0.750	0.743	0.895	0.601	0	2	0	0	26.73	0	37.85	48.43	63.37
23	0.171	0.788	0.750	0.745	0.899	0.590	0	2	0	0	19.26	0	37.97	49.57	63.58
28	0.171	0.788	0.750	0.748	0.900	0.576	0	1	0	0	5.530	0	38.11	51.38	63.82
33	0.171	0.788	0.750	0.001	-1.130	-3.878	0	1	0	0	9.894	0	38.08	50.84	63.78
38	0.171	0.788	0.750	0.751	0.901	0.563	0	1	0	0	13.66	0	38.24	50.63	64.05
43	0.171	0.788	0.750	0.750	0.900	0.564	0	1	0	0	17.45	0	38.23	50.17	64.03
48	0.171	0.788	0.750	0.750	0.899	0.566	0	1	0	0	21.87	0	38.21	49.62	64.01
53	0.171	0.788	0.750	0.754	0.896	0.549	0	0	0	0	0.000	0	38.38	52.35	64.29
Bucholtz	0.171	0.788	0.750	0.746	0.898	0.588	0	2	0	0	22.98	0	37.99	49.11	63.61
Kawagutchi	0.171	0.788	0.750	0.746	0.899	0.587	0	2	0	0	21.10	0	38.00	49.35	63.62
Quetin	0.171	0.788	0.750	0.753	0.900	0.550	0	1	0	0	11.18	0	38.37	51.09	64.27
Tarling J	0.171	0.788	0.750	0.740	0.897	0.613	0	2	0	0	21.66	0	37.73	48.93	63.16
Tarling FS	0.171	0.788	0.750	0.742	0.893	0.613	0	3	0	0	29.20	0	37.84	48.12	63.16
Tarling FA	0.171	0.788	0.750	0.727	-0.725	0.576	0	3	0	0	30.36	0	37.09	47.59	63.83
Filtration rate <25 mm intercept															
0.0007	0.125	—	—	0.391	—	—	0	—	—	0	—	—	32.74	—	—
0.000775	0.148	—	—	0.550	—	—	0	—	—	0	—	—	35.10	—	—
0.00085	0.171	—	—	0.749	—	—	0	—	—	0	—	—	37.47	—	—
0.000925	0.194	—	—	0.994	—	—	0	—	—	0	—	—	39.86	—	—
0.001	0.217	—	—	1.292	—	—	0	—	—	0	—	—	42.27	—	—
Filtration rate <25 mm slope															
0.65	0.071	—	—	0.099	—	—	0	—	—	0	—	—	26.21	—	—
0.7375	0.115	—	—	0.256	—	—	0	—	—	0	—	—	30.65	—	—
0.825	0.171	—	—	0.749	—	—	0	—	—	0	—	—	37.47	—	—
0.9125	0.242	—	—	2.773	—	—	1	—	—	5.103	—	—	48.46	—	—
1	0.333	—	—	16.54	—	—	1	—	—	58.80	—	—	70.00	—	—
Filtration rate >36 mm intercept															

Parameter	Growth rate at day 2 (mg carbon day^{-1})			Growth rate at day 100 (mg carbon day^{-1})			Number of spawns			Total carbon spawned (mg carbon)			Length at day 100 (mm)		
	20	40	60	20	40	60	20	40	60	20	40	60	20	40	60
0.002	—	0.270	-0.293	—	0.244	-0.132	—	0	0	—	0	0	—	43.95	57.24
0.002715	—	0.529	0.228	—	0.543	0.203	—	1	0	—	4.607	0	—	47.42	60.50
0.00343	—	0.788	0.750	—	0.900	0.588	—	1	0	—	14.89	0	—	50.12	63.61
0.004145	—	1.048	1.271	—	1.309	1.020	—	2	0	—	27.04	0	—	52.65	66.58
0.00486	—	1.307	1.793	—	1.77	1.499	—	2	1	—	40.04	2.265	—	55.14	69.31
Allocation to reproduction															
0.3	0.171	0.788	0.750	0.746	0.899	0.588	0	0	0	0	0	0	37.99	51.83	63.61
0.4	0.171	0.788	0.750	0.746	0.899	0.588	0	0	0	0	0	0	37.99	51.83	63.61
0.5	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	2.687	0	37.99	51.54	63.61
0.6	0.171	0.788	0.750	0.746	0.901	0.588	0	1	0	0	8.549	0	37.99	50.87	63.61
0.7	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
0.8	0.171	0.788	0.750	0.746	0.898	0.588	0	2	0	0	21.82	0	37.99	49.26	63.61
0.9	0.171	0.788	0.750	0.746	0.895	0.588	0	2	0	0	28.32	0	37.99	48.41	63.61
1	0.171	0.788	0.750	0.746	0.889	0.588	0	2	0	0	34.75	0	37.99	47.52	63.61
Swimming cost															
0.3	0.179	0.867	1.064	0.839	1.067	0.893	0	2	0	0	29.02	0	39.10	49.99	65.58
0.4	0.175	0.828	0.907	0.791	0.988	0.733	0	2	0	0	16.75	0	38.55	50.68	64.59
0.5	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
0.6	0.167	0.749	0.592	0.703	0.818	0.456	0	1	0	0	12.57	0	37.43	49.64	62.65
0.7	0.163	0.710	0.435	0.664	0.741	0.337	0	1	0	0	11.01	0	36.88	49.09	61.70
0.8	0.159	0.671	0.278	0.627	0.668	0.229	0	1	0	0	9.107	0	36.32	48.60	60.77
0.77 mg O_2 mg DM^{-1} h^{-1}	0.082	0.180	-1.594	0.164	0.112	-0.438	0	0	0	0	0.000	0	27.92	41.99	50.75
Feeding															
0.005	0.171	0.789	0.753	0.747	0.900	0.592	0	2	0	0	23.03	0	38.01	49.12	63.63
0.01	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
0.015	0.171	0.787	0.746	0.744	0.899	0.585	0	1	0	0	14.86	0	37.97	50.11	63.59
0.02	0.171	0.787	0.743	0.743	0.900	0.582	0	1	0	0	14.84	0	37.95	50.09	63.56
0.025	0.170	0.786	0.739	0.742	0.895	0.578	0	1	0	0	14.81	0	37.93	50.08	63.54
0.03	0.170	0.785	0.735	0.741	0.893	0.575	0	1	0	0	14.78	0	37.91	50.06	63.52
0.035	0.170	0.784	0.731	0.739	0.891	0.572	0	1	0	0	14.76	0	37.89	50.05	63.50
0.04	0.170	0.783	0.728	0.738	0.889	0.569	0	1	0	0	14.73	0	37.87	50.04	63.47
0.045	0.169	0.782	0.725	0.737	0.887	0.565	0	1	0	0	14.71	0	37.84	50.02	63.45

Parameter	Growth rate at day 2 (mg carbon day^{-1})			Growth rate at day 100 (mg carbon day^{-1})			Number of spawns			Total carbon spawned (mg carbon)			Length at day 100 (mm)		
	20	40	60	20	40	60	20	40	60	20	40	60	20	40	60
0.055	0.169	0.780	0.717	0.734	0.884	0.559	0	1	0	0	14.65	0	37.80	49.99	63.41
0.06	0.169	0.779	0.714	0.733	0.882	0.556	0	1	0	0	14.01	0	37.78	50.05	63.38
0.065	0.168	0.779	0.710	0.732	0.880	0.553	0	1	0	0	13.98	0	37.76	50.04	63.36
0.07	0.168	0.777	0.707	0.731	0.878	0.549	0	1	0	0	13.96	0	37.74	50.02	63.34
0.075	0.168	0.776	0.703	0.729	0.876	0.546	0	1	0	0	13.93	0	37.72	50.01	63.32
0.08	0.168	0.775	0.699	0.728	0.874	0.543	0	1	0	0	13.91	0	37.70	50.00	63.29
0.085	0.167	0.774	0.696	0.727	0.872	0.540	0	1	0	0	13.88	0	37.68	49.98	63.27
0.09	0.167	0.773	0.692	0.726	0.871	0.537	0	1	0	0	13.86	0	37.66	49.97	63.25
0.095	0.167	0.772	0.689	0.725	0.869	0.534	0	1	0	0	13.83	0	37.63	49.95	63.23
0.1	0.166	0.771	0.685	0.723	0.867	0.530	0	1	0	0	13.81	0	37.61	49.94	63.20
Moult cost															
1.4	0.171	0.788	0.750	0.747	0.898	0.582	0	2	0	0	22.99	0	38.05	49.19	63.71
1.6	0.171	0.788	0.750	0.745	0.900	0.589	0	1	0	0	14.89	0	37.98	50.11	63.60
1.8	0.171	0.788	0.750	0.744	0.900	0.595	0	1	0	0	14.88	0	37.92	50.02	63.48
2	0.171	0.788	0.750	0.743	0.900	0.602	0	1	0	0	14.87	0	37.85	49.93	63.37
2.2	0.171	0.788	0.750	0.741	0.900	0.608	0	1	0	0	14.86	0	37.79	49.84	63.25
2.4	0.171	0.788	0.750	0.740	0.900	0.614	0	1	0	0	15.48	0	37.72	49.67	63.14
2.6	0.171	0.788	0.750	0.739	0.899	0.620	0	1	0	0	15.47	0	37.65	49.58	63.02
Ovary size															
0.2	0.171	0.788	0.750	0.727	0.881	0.639	1	3	1	3.3503	41.75	14.63	37.11	46.51	62.64
0.3	0.171	0.788	0.750	0.746	0.896	0.588	0	2	0	0	26.72	0	37.99	48.61	63.61
0.4	0.171	0.788	0.750	0.746	0.899	0.588	0	2	0	0	20.86	0	37.99	49.38	63.61
0.5	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	14.89	0	37.99	50.12	63.61
0.6	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	9.363	0	37.99	50.78	63.61
0.7	0.171	0.788	0.750	0.746	0.900	0.588	0	1	0	0	3.763	0	37.99	51.42	63.61
0.8	0.171	0.788	0.750	0.746	0.899	0.588	0	0	0	0	0.000	0	37.99	51.83	63.61
0.9	0.171	0.788	0.750	0.746	0.899	0.588	0	0	0	0	0.000	0	37.99	51.83	63.61

APPENDIX H. CHLOROPHYLL- AND TEMPERATURE-DEPTH PROFILES FROM JR70

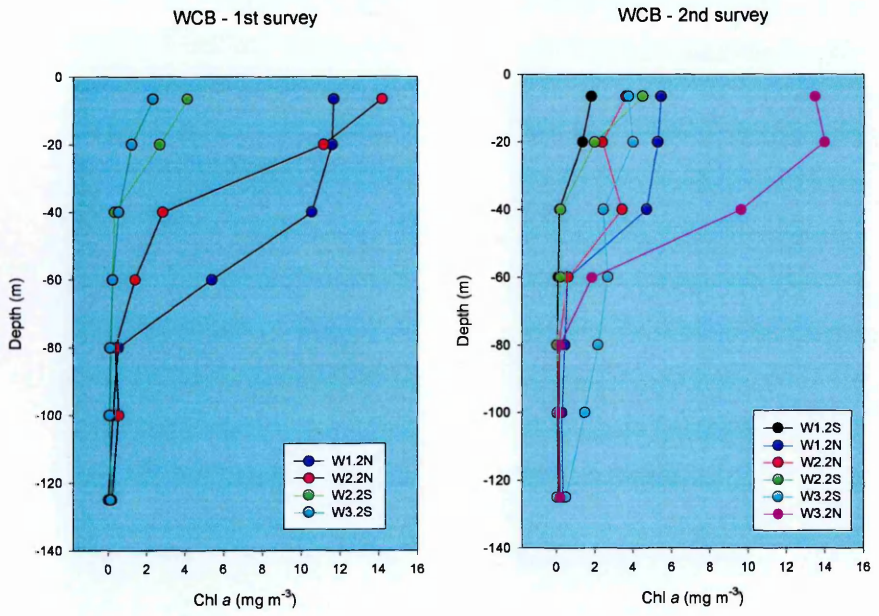

Figure 1. Chlorophyll *a* variation with depth at stations in the Western Core Box (WCB) sampled during JR70. Figure from Ward et al. (2002).

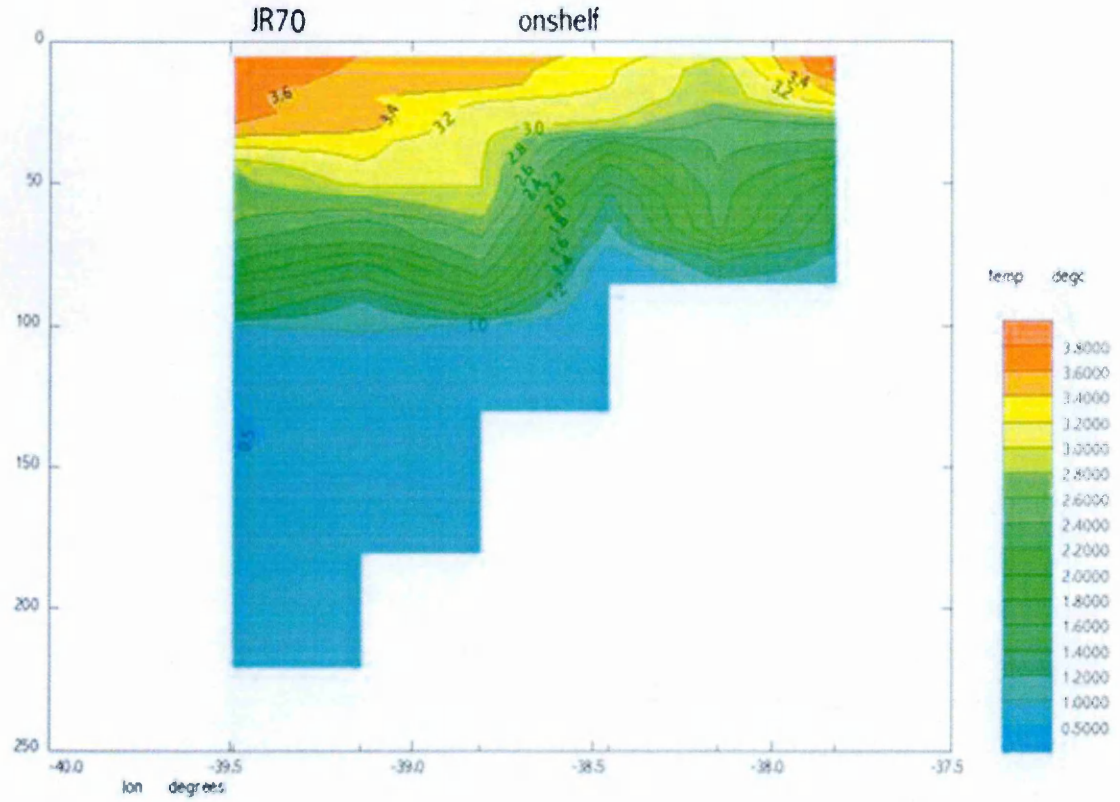

Figure 2. Temperature stratification with depth along the on-shelf transect of the WCB during JR70. Figure from Ward et al. (2002).

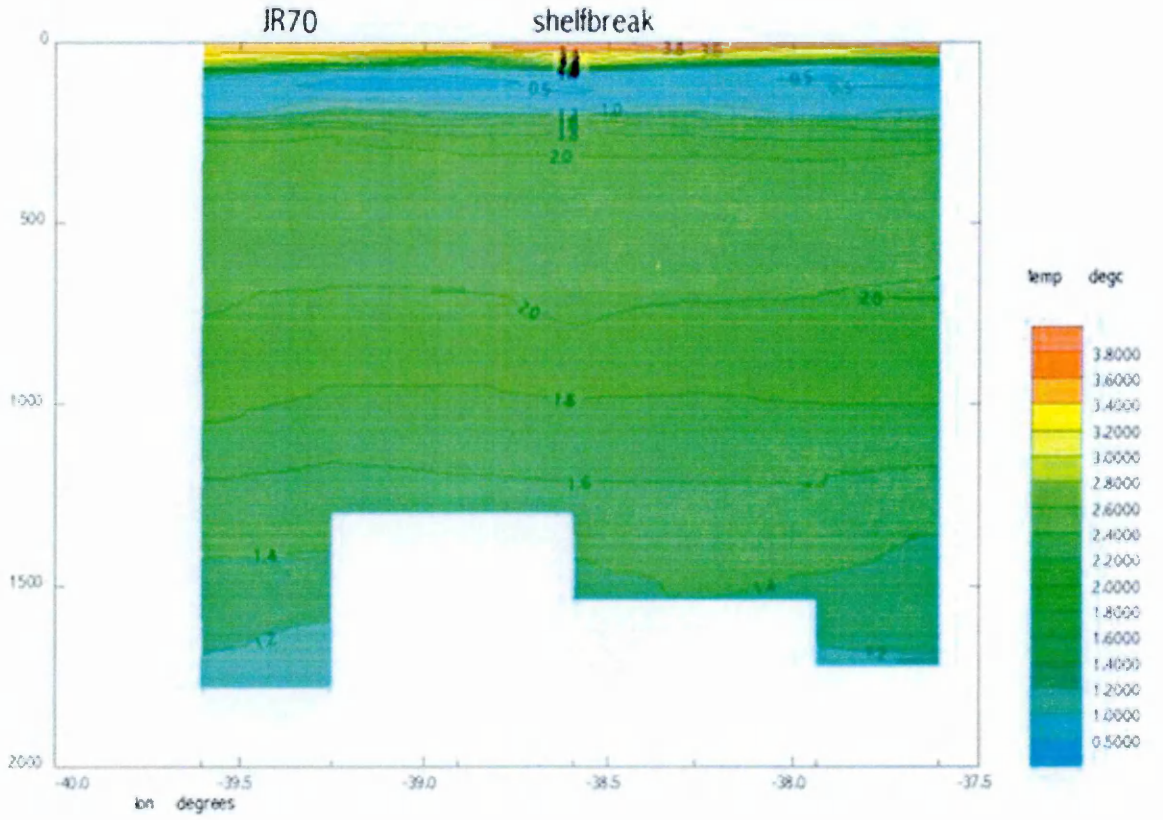

Figure 3. Temperature stratification with depth along the shelf-break transect of the WCB during JR70. Figure from Ward et al. (2002).

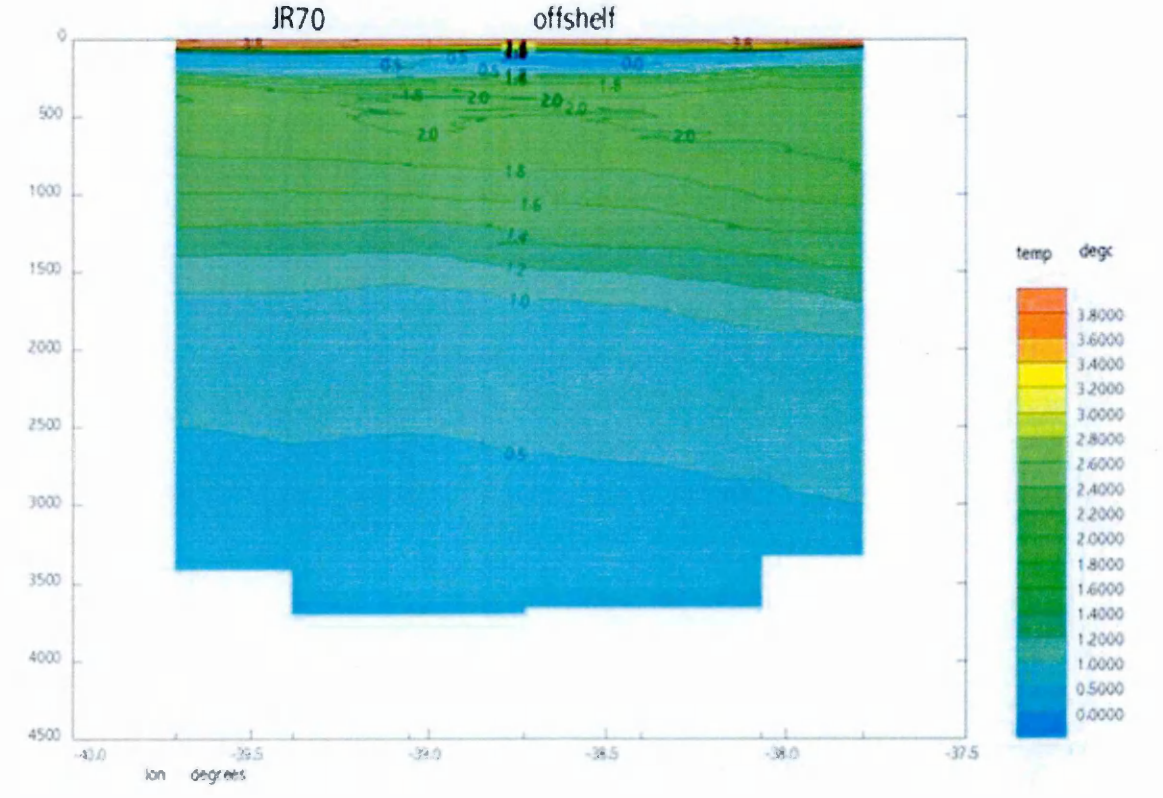

Figure 4. Temperature stratification with depth along the off-shelf transect of the WCB during JR70. Figure from Ward et al. (2002).